PHARMACOLOGICAL and OTHER STUDIES,

submitted for the degree of

DOCTOR OF MEDICINE

by

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PUBLICATIONS.

Of the publications submitted, those numbered 2,5,11,12,16,28 and 30 are the candidate's own work. He is also the author of numbers 4,6,7,8,9,10,13,14,15,17, 19,21,25 and 29, and although most of the work has been carried out in collaboration, it is hoped that credit can be allowed for it up to a point, since many of the investigations are consecutive and several are scarcely suitable for single-handed study.

STUDIES ON THE ACTIONS OF POST-PITUITARY EXTRACTS.

(1) Studies on the Pituitary, lV. Quantitative Comparison of Pressor Activity. (With L.T. Hogben and W. Schlapp). Quart. J. Exper. Physiol.1924, XlV, p.301-318.

(2) Action of Pituitary Extracts on Intestinal Muscle. Quart. J. Exper. Physiol.1925,XV, p. 191 - 200.

(3) Action of Extracts of Posterior Lobe of the Pituitary Body on the Pulmonary Circulation. (With the late Sir E.Sharpey Schafer). Quart. J. Exper. Physiol. 1926.XVl, p. 251 - 280.

(4) Action of Pituitary Extracts upon Isolated Blood-Vessels.(With E.D.Portman). J. Physiol. 1928. LXV. (Proc.)

(5) The Action of Pituitary Extracts on the Kidney. Quart. J. Exper. Physiol. XXlll,1933, p. 319 - 333.

(6) Actions of Posterior Pituitary Principles on the Colon. (With H.L. Settle). J. Physiol.LXXXVl. (Proc). 1935.

STUDIES ON THE ACTIONS OF LOCAL ANAESTHETICS.

(7) The Pharmacology of Percaine.
(With M.C.G. Israëls).
British Medical Journal, November 1931.

(8) The Estimation of Relative Toxicities and Relative Efficiencies of Local Anaesthetics.
(With M.C.G. Israëls).
J. Pharmacol. and Exper. Therap. XLIV. 1932, p. 361 - 368.

(9) Observations on Experimental Spinal Anaesthesia. (With E.F. Hill).
J. Pharmacol. and Exper. Therap. XLVII, 1933, p. 151 - 162.

(10) The Action of Local Anaesthetics on the Respiratory Apparatus.
(With E.F. Hill).
J. Pharmacol. and Exper. Therap. LII. 1935, p. 454 - 464.

(11) Neurological Sequelae of Spinal Anaesthesia. Experimental and Pharmacological Considerations.
Proceedings of Royal Society of Medicine, Vol. XXX. 1937.

(12) Local Anaesthetics on Blood-Vessels.
The Lancet, June, 1937. (letter).

(13) The Fate of Drugs used in Spinal Anaesthesia.
(With K. Bullock).
J. Pharmacol. and Exper. Therap. 62, 1938. p. 39 - 53.

(14) Observations on Dental Local Anaesthetic Solutions.
(With S.L. Wilson and K. Bullock).
British Dental Journal, 64, March, 1938.

(15) An Experimental Investigation into the Cause of Paralysis Following Spinal Anaesthesia.
(With K.H. Watkins).
British Journal of Surgery, April 1938.
(Reprint not yet available).

STUDIES ON THE ACTIONS OF ADRENALINE AND THE CONTROL OF CERTAIN VISCERA.

(16) Action of Adrenaline on the Perfused Fish Heart.
Quart. J. Exper. Physiol. 1925, XV.
p. 69 - 80.

(17) Adrenaline Vaso-dilatation. (With W. Schlapp)
J. Physiol. 1926, LXl (Proc.)

(18) Action of Drugs upon the Movements of the Stomach.
(With E.D.McCrea).
Quart. J. Exper. Physiol. 1928. XlV.
p. 161 - 170.

(19) Observations on the Control of the Bladder - The Effects of Nervous Stimulation and of Drugs.
(With E.D.McCrea).
Quart. J. Exper. Physiol. 1930. XX.
p. 379 - 391.

(20) Pre-sacral Sympathectomy and the Urinary Bladder.
(With E.D. McCrea).
British J. of Urology. Vol. Vl, 1934,
p. 119 - 127.

(21) Pre-sacral Sympathectomy and the Bladder in Man.
(With E.D.McCrea).
Journal of Physiology, LXXXVl, 1935. (Proc.)

UNCLASSIFIED

(27) Action of the Intercostal Muscles.
(With the late Sir E.Sharpey Schafer).
J. Physiol. 1925, Vol. LX.(proc.)

(28) An Improved Teat for Infants'
Feeding Bottles.
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(29) Assay of Digitalis by the Cat Method.
(With W. Schlapp).
Quart. J. Pharm. and Pharmacol. 1930.
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(30) The Assay of Strophanthus Preparations
by the Cat Method.
Quart. J. Pharm. and Pharmacol.Vol.V11
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(31) Observations on Experimental Shock.
(With R.I. Holt).
British Medical Journal, June,1934.

STUDIES ON THE PITUITARY.—IV. QUANTITATIVE COMPARISON OF PRESSOR ACTIVITY.

By LANCELOT T. HOGBEN, WALTER SCHLAPP, and A. D. MACDONALD. From the Physiology Department, Edinburgh University. (With eight figures.)

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CONTENTS.

1. INTRODUCTION.

THE object of the present communication is to indicate certain refinements by which the method of standardisation of pressor activity suggested by DALE and LAIDLAW (1912) may be made more satisfactory for the purpose of biochemical investigations. It may be—both as regards accuracy and general appropriateness to the purpose for which such extracts are employed in clinical practice—that the uterus test of the same authors has afforded a preferable method of assay for pituitary preparations. But, apart from the use of pituitary extract for clinical purposes other than of an obstetrical nature, an improved procedure for comparing the pressor activity of different samples in researches on the active principles of the pituitary gland is evidently desirable. Without reviewing the extensive literature concerned with this subject, it will suffice to recall the two pre-eminent difficulties which beset pressor standardisation. These are: (*a*) the possible participation of more than one component of the vasomotor activity of pituitary extract, and (*b*) the diminution of response to successive doses.

2. THE DEPRESSOR COMPONENT.

Depressor action of pituitary extracts was first observed by SCHAFER and VINCENT (1899), who indicated a method by which a preparation evoking a purely pressor response might be made. SCHAFER and VINCENT found that in the anæsthetised dog a second injection administered shortly after the first produces a fall unaccompanied by any

appreciable rise in blood-pressure. DALE and LAIDLAW (1912), using the spinal cat, experienced no such effect as that recorded by SCHAFER and VINCENT : in their observations a slight fall is seen to precede the diminished rise which follows repeated injections, but a pure depressor action is not recorded. " It may be doubted," they state, " whether the depressor action seen with injections subsequent to the first is wholly due to a different principle. PATON and WATSON have recently shown that in the duck a fall of blood-pressure is the characteristic effect of the extract." This conclusion appears to the present writers to rest on a failure to appreciate the very fundamental difference between the response of the spinal and anæsthetised carnivor. We have elsewhere shown (HOGBEN and SCHLAPP, 1924) : (*a*) that in the anæsthetised cat pituitary extract unextracted with alcohol if administered within the period of complete tolerance for the pressor principle produces a pure depression (*cf.* fig. 1, A) ; (*b*) that similar treatment in the case of a spinal animal with low blood-pressure (45–70 mm.) evokes a preliminary fall followed by an evanescent rise (fig. 1, B) ; (*c*) that both these depressor effects can be obliterated by alcoholic extraction ; (*d*) that histamine behaves in a similar way to the pituitary depressor substance by giving a less pronounced depressor effect followed by a quite considerable rise in blood-pressure rather than a predominantly depressor response in the spinal cat ; (*e*) that the substance responsible for the consistently depressor action of pituitary extract on the avine circulation is not the same as that which produces depression in the carnivor, nor is it histamine.

The relevance of these considerations for a refinement of the method of pressor standardisation may be appreciated from the following quotation from DALE and LAIDLAW'S paper : " The difficulty of the declining effect of successive doses may to a certain extent be overcome. If a cat with the cord cut in the neck and the brain destroyed be used, no injection being made till, under the artificial respiration, all anæsthetic has been removed, and the blood-pressure has become low and steady, the first dose has an enormously greater effect than the second, while the third has yet smaller result. But if the doses given are small (not more than 0·1 c.c. of a 10 per cent. decoction of fresh tissue), it will be found that after about the fourth dose the effects of subsequent doses though small are almost uniform." Clearly, if the same substance which produces the depressor effect on the anæsthetised carnivor evokes a rise in blood-pressure in the spinal animal, it is evident that such a procedure might in reality be measuring the depressor action of pituitary extract. If, moreover, it can be shown that a chemically homogeneous substance, namely histamine, can produce a correspondingly differential effect on the anæsthetised and spinal cat, any *à priori* obstacle to accepting the conclusion that the depressor substance of SCHAFER and VINCENT exerts a pressor action upon the

decerebrate preparation is removed. When we bear in mind that histamine is produced in animal tissues after death, and the extreme potency of its action on the circulatory system, it is evident that an indispensable prerequisite to a rational method of quantitative comparison of pressor activity is the removal of the depressor component.

Since the publication of the paper mentioned above we have carried out further experiments comparing the action of histamine and acetyl choline on the etherised and spinal cat, and of pituitary extract on a corresponding series of pairs. As we have quoted protocols in detail elsewhere, we may here simply summarise our later observations as follows :—

1. Acetyl choline and histamine both produce a pure fall in blood-pressure on the anæsthetised cat (urethane or ether); but, whereas the former never produces other than a depressor effect on the spinal preparation, histamine—consistently with DALE and RICHARD'S analysis of its double seat of action—evokes in the spinal cat, after a rapidly evanescent depression, a pronounced rise of blood-pressure which may be equal to or several times greater than the preliminary fall. This pressor action of histamine in the spinal animal is augmented by previous administration of pituitary extract.

2. The depressor action of pituitary extracts is qualitatively similar in different individuals prepared in the same way, when the same samples in doses of equivalent magnitude are administered at corresponding time intervals. The actual predominance of the depressor over the pressor effect after the first injection depends on the condition of the animal, whether anæsthetised or decerebrate, the actual sample of pituitary extract employed, and the time interval between the doses. It is in fact bound up with the degree of tolerance to the pressor substance as described below. The salient conclusions to which we have been led may be epitomised thus: The extent of depressor action varies very much with different preparations; in all the commercial extracts which we have tested a purely depressor effect was produced after one or more injections, depending on the dosage, at suitable time intervals; but a pure depressor response was in no circumstances obtained from the spinal cat with a blood-pressure of 40–70 mm. The accompanying illustrations (figs. 1, A and 1, B) display the very characteristic contrast between the action of histamine and of successive pituitary injections upon the anæsthetised and spinal cat.

As to whether there is any depressor effect obtained from absolutely fresh extracts, we are not at present in a position to say, though we have prepared depressor free pituitary substance from glands plunged into cold acetone within a few minutes of killing. There can be little doubt that the depressor action of commercial samples differs prodigiously. And in manufacture on a large scale it may not be possible to avoid this. Since, however, six hours' extraction with ethyl alcohol usually suffices

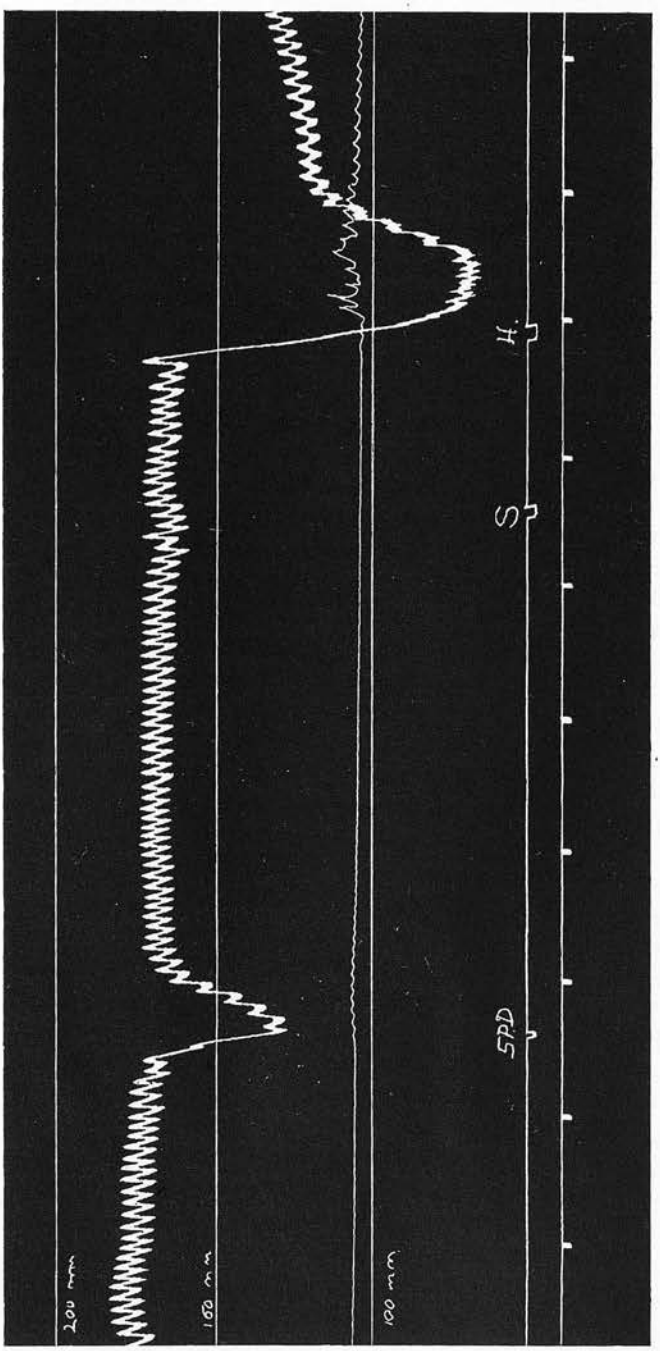

FIG. 1, A.—Cat urethane. Showing effect of 0·5 c.c. Parke Davis pituitrin after several previous injections (P.D.), and of 0·1 mg. histamine phosphate (H.), 1 c.c. saline. Time interval one minute.

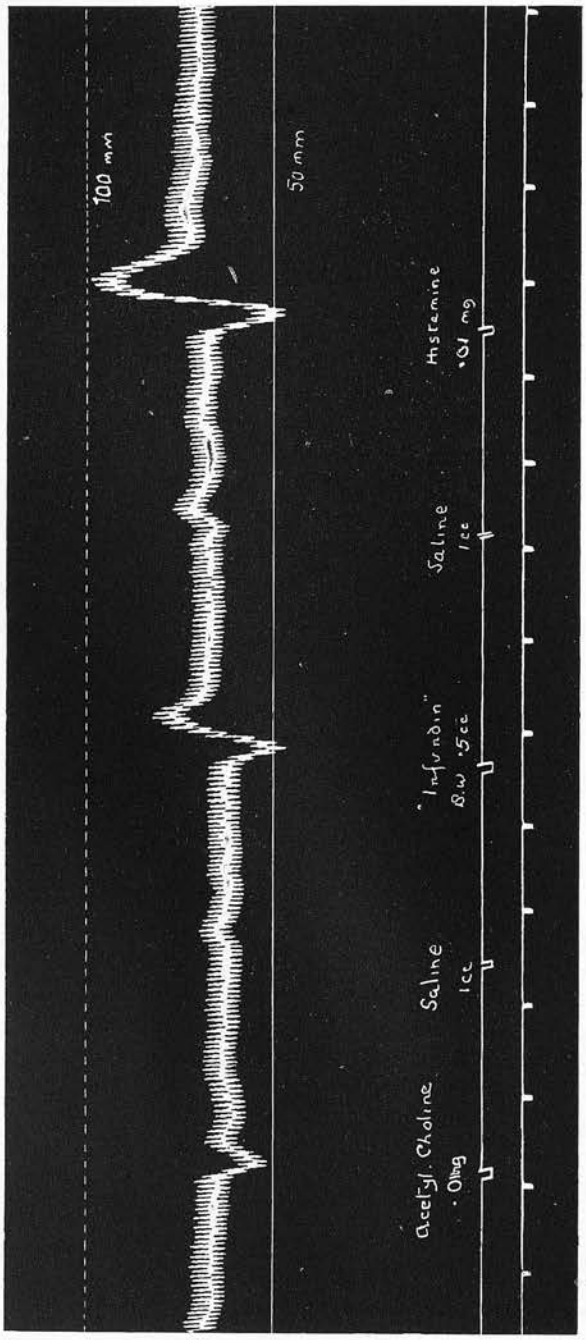

FIG. 1, B.—Spinal cat. Showing effect of 0·01 mg. acetyl choline, 0·5 c.c. Burroughs Wellcome's infundin after several previous injections, and 0·01 mg. histamine phosphate. Time interval one minute.

to eliminate the depressor substance, it would seem eminently desirable that extracts for clinical or biochemical purposes in any way concerned with pressor action should be subjected to previous alcoholic treatment. There should be no need after what has been said above to draw attention to the fallacy of selecting for standardisation one species of animal rather than another on the ground that it is less susceptible to the depressor substance. The experiments which follow were in all cases carried out with depressor free extracts.

3. Tolerance in Relation to the Time Interval.

In attempting the pressor assay of pituitary extract there are obvious objections to using the anæsthetised dog as prescribed by Hamilton in preference to the spinal cat as employed by Dale and Laidlaw. The anæsthetised animal is far less sensitive than the spinal preparation with a low blood-pressure. Further, the cat is less costly and the brain is more readily destroyed. The published records of Hamilton's method suffice to indicate what measure of accuracy may be expected from the procedure he advocates. With proper insufflation we find it possible to keep the spinal cat with a blood-pressure that does not deviate from the level attained after a rest of about an hour by more than 5 mm. during fifteen to twenty hours.

The essential difference between other methods of comparing the pressor activity of pituitary extracts and the procedure which we have explored, lies in the fact that other workers have matched the test sample with the standard on a diminishing or diminished series of responses as compared with that which results from the first injection. If the matching is performed at short intervals after the response has diminished to a more or less constant level, as Dale and Laidlaw proposed and as Burn and Dale have apparently adopted, it is doubtful whether, when commercial preparations are used, the real pressor substance contributes appreciably to the effects observed. When alcohol-extracted, " depressor-free " preparations are employed, the diminution of response, though most obvious after the first, second, and third injections, is more or less continuous till complete immunity is attained, providing that the time interval is relatively short. Now the sources of error in matching on a descending curve are evident enough, but in any case this method leaves a very small margin of response on which to work. It is thus clearly desirable to obtain further knowledge of the acquired tolerance to successive injections with a view to adjusting the dosage and time interval to meet the requirements of an appropriate regularity and magnitude of response.

The result of our experiments has been to show that after a lapse of time, varying with the size of the dose given, full response can be recovered when complete immunity has been produced by repeated injections ;

and it became evident, at an early stage in the investigation, that by administering the extract only at the end of the period adequate for complete recovery, a constant response can be obtained over periods of many hours. The following protocol is typical of these preliminary experiments :—

Spinal Cat.—Operation at 10 a.m.

1.20 p.m.	systolic (carotid) pressure		52 mm.
2. 0 ,,	4 mg. dried, depressor-free ox posterior lobe injected (jugular vein) : pressure rises from	50–124 mm.	74 ,,
2.15 ,,	same dose repeated pressure rises from	54–89 ,,	35 ,,
2.25 ,,	,, ,, ,, ,,	64–83 ,,	19 ,,
2.35 ,,	,, ,, ,, ,,	64–78 ,,	14 ,,
2.45 ,,	,, ,, ,, ,,	60–68 ,,	8 ,,
2.55 ,,	,, ,, ,, ,,	60–68 ,,	8 ,,
3. 0 ,,	,, ,, ,, ,,	60–63 ,,	3 ,,
3. 5 ,,	,, ,, ,, ,,	60–62 ,,	2 ,,

(The response to the last two doses being of the order of change produced by saline injection, complete tolerance was attained at 3 o'clock.)

5. 5 p.m.	same dose repeated : pressure rises from	60–126 mm.	66 mm.
5.35 ,,	,, ,, ,, ,,	63– 86 ,,	23 ,,
7.26 ,,	,, ,, ,, ,,	54–128 ,,	74 ,,
8.26 ,,	,, ,, ,, ,,	54–110 ,,	56 ,,
10. 0 ,,	,, ,, ,, ,,	54–127 ,,	73 ,,

It is to be noted that whereas the period of an hour and a half was required to restore the original response at the end of the experiment, a two-hours' period was not sufficient after the succession of doses given within the first hour, thus suggesting that the amount actually circulating, and therefore possibly the rate of excretion, enters, as DALE (1909) first suggested, into the question as to how long a period is adequate for the recovery of maximum reactivity.

Experience showed that this dose was maximal. Working with a sub-maximal dose, an hour was always found sufficient for the maintenance of constant response. Experiments carried out over periods of eight to ten hours encouraged the hope that consistent discrimination of 10 or 15 per cent. differences could be obtained (fig. 2) when the injections were made at hourly intervals.

In considering how the greatest refinement of discrimination may be obtained, it is necessary to take into account the relation between increase in pressure and varying dosage, since the former manifestly cannot be a linear function of the latter. The form of the curve which expresses this relation is highly instructive from this standpoint. Since

the gradient (fig. 3) is steepest in the middle portion, a high degree of discrimination may be expected by working around a value roughly equivalent to one-half the dose required to produce a maximum response. Clearly, then, the standard dose should correspond as nearly as possible to the point of inflexion on the curve, since the greatest discrimination will be obtained between doses round about the value for which dp/dx is a maximum. We compare the actual increment of pressure, not the height obtained, and it is most convenient to consider the increment from the upper level of a respiration wave. In general, a quantity of extract adequate to produce a rise in pressure of about 55 mm. is the standard which in our experience it is desirable to set.

We have carried out a series of experiments in which from 11 to 15

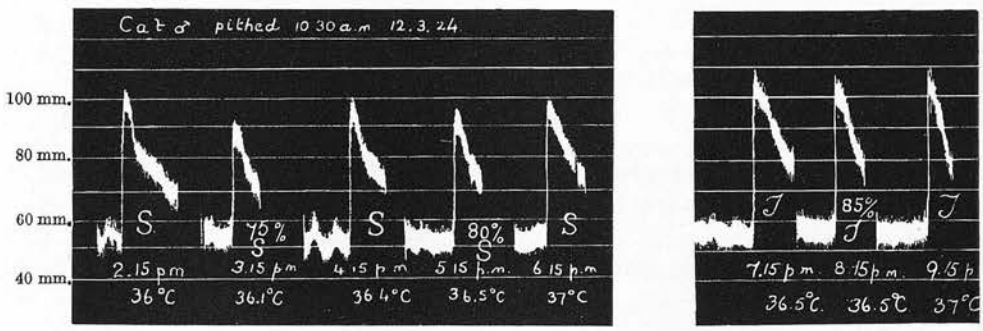

FIG. 2.—Eight consecutive injections of pituitary extract at hourly intervals on spinal cat, showing 25 per cent., 20 per cent., and 15 per cent. discrimination. Slow drum (2 mm. per minute). Lined in spaces of 10 mm. pressure, lower limit 40 mm., systolic pressure about 60 mm. throughout. S and T are different standards of depressor free extract.

successive doses of a standard and a known dilution of the standard were alternately administered to show that discrimination up to 10 per cent. differences can be consistently maintained for periods which permit 6 to 8 pairs of matches to be made. The method of producing the spinal animal was that employed in DALE'S laboratory and kindly demonstrated to one of us by Dr J. H. BURN. The cord is exposed under deep anæsthesia by chipping off the neural arch of the axis vertebra, the aperture in which is plugged with plasticine after transection of the cord and destruction of the brain. The first injection is not made till two or three hours later. Without recourse to a thermostat, a constant body temperature may be maintained by wrapping the animal in cotton-wool on a warm table. A rectal temperature of 35–37° C. was thus maintained for twenty hours after the above operation. If properly inflated—and special importance is to be attached to the degree of insufflation—a cat prepared in this way will maintain a blood-pressure of 80 mm. for twenty-four hours. An experienced worker will be able

to regulate the final pressure by the amount of bleeding permitted in the destruction of the brain. It is more satisfactory to work with a basal pressure of about 60 mm. The cannula should be cleared with a feather between each injection of pituitary extract, the remnant of

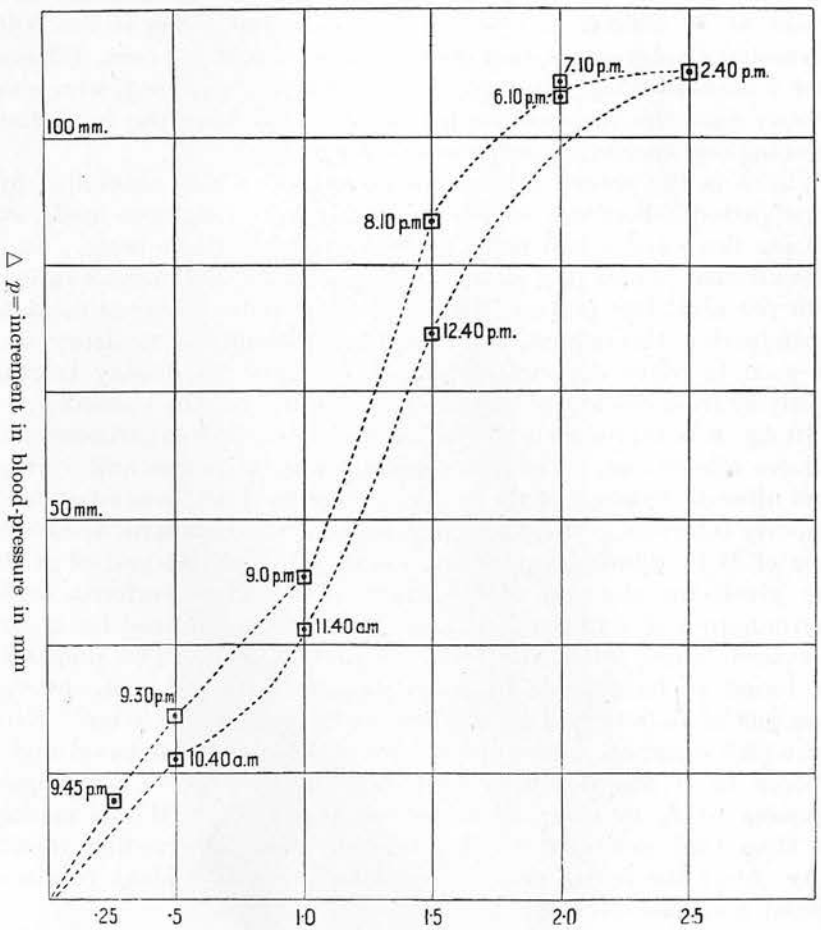

FIG. 3.—Graph showing increment of pressure with varying doses (hourly intervals). Ordinate *rise in* pressure. Abscissa dosage in milligrams desiccated substance alcohol-extracted ox posterior lobe.

which should immediately be washed into the vein by 0·5 c.c. saline. In our experience the jugular is preferable to the femoral for injection; the head being dead, there is less likelihood of obtaining vasomotor reflexes in manipulating the vein tube.

In comparing the effect of successive doses, the upper limit of blood-pressure at the actual moment of injection should be noted, so that

where fine shades of discrimination are required the increment can be measured with a pair of dividers. In fig. 4 is seen the effect of 11 successive doses of pituitary extract at hourly intervals, commencing two and a quarter hours after destruction of the brain. The experiment was discontinued at 10.15 p.m., but the cat was still reactive to the extract at 10 o'clock on the following morning, when it was killed. This record displays consistent discrimination of a 25 per cent. difference. After 2 doses of 2 mg., alternate doses of 1·5 mg. and 2 mg. were given; in every case the response to 1·5 mg. was less than the immediately preceding and succeeding responses to 2 mg.

Fig. 5 is the record of an experiment which was continued for a longer period. Fourteen successive hourly injections were made, commencing three and a half hours after destruction of the brain. In this series alternate doses of a standard (2 mg.) and of a dilution equivalent to 20 per cent. less (1·6 mg.) were administered. There is consistent discrimination throughout the experiment, despite a tendency which was seen in other experiments, for the animal to display increased sensitivity from the seventh to the ninth hour after the operation.

In fig. 6 is reproduced the record of the longest experiment which we have carried out. The first injection was made two and a quarter hours after destruction of the brain. In all, 16 doses were administered at hourly intervals. After two injections of the standard A, alternate doses of B (a dilution equivalent to 85 per cent. A) and of A itself were given for the rest of the experiment. Here perfectly evident discrimination of a 15 per cent. difference was maintained for a period of sixteen hours, when the test was discontinued. The preparation was found to be capable of discriminating a 10 per cent. difference when one of us returned to the laboratory eight hours later. Except at the fifth injection, where the sensitivity obviously increased and the response to B was decidedly less than the average of the adjacent responses to A, in every case the rise produced by B was markedly less than that produced by the preceding and succeeding injection of A. After the fourth dose the discrimination is evident to the eye without finer measurement.

In the last experiment it will be noted that the quality of the discrimination improves towards the end of the experiment. This is of course propitious for standardisation. Wherever we have tried at the end of a series where the response is maintaining a tolerably constant level (fig. 7), we have obtained 10 per cent. discrimination: fig. 8 shows discrimination of a 7 per cent. difference at this stage. We have not carried out any test with a 10 per cent. difference for so long a period as in the foregoing experiment, but have performed several in which equally clear discrimination is seen in three or four succeeding pairs.

Such experiments, as well as the form of the curve in fig. 3, show that there is no insuperable obstacle to quantitative comparison of pressor

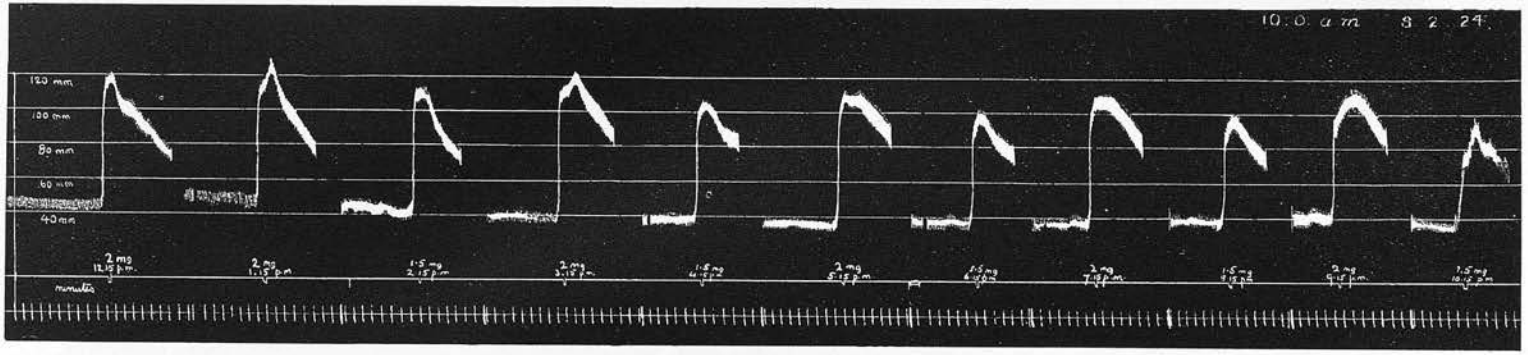

FIG. 4.—Spinal cat. Showing the effect of eleven successive doses of pituitary extract at hourly intervals commencing at 12.15 p.m., and showing consistent discrimination between doses differing by 25 per cent. administered alternately after two injections of the standard. The operation was performed at 10.0 a.m. The time interval (bottom line) is one minute.

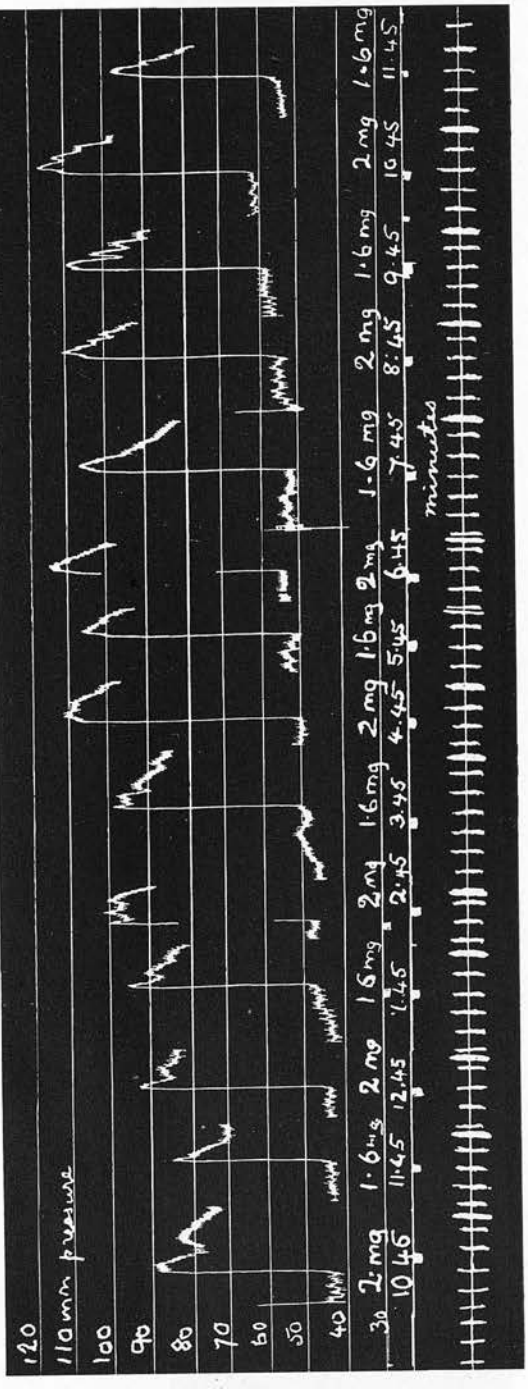

FIG. 5.—Spinal cat. Fourteen successive doses of pituitary extract at hourly intervals, alternate doses differing by 20 per cent. Operation at 7.15 a.m. : first injection 10.45 a.m. Time interval (bottom line) one minute as before.

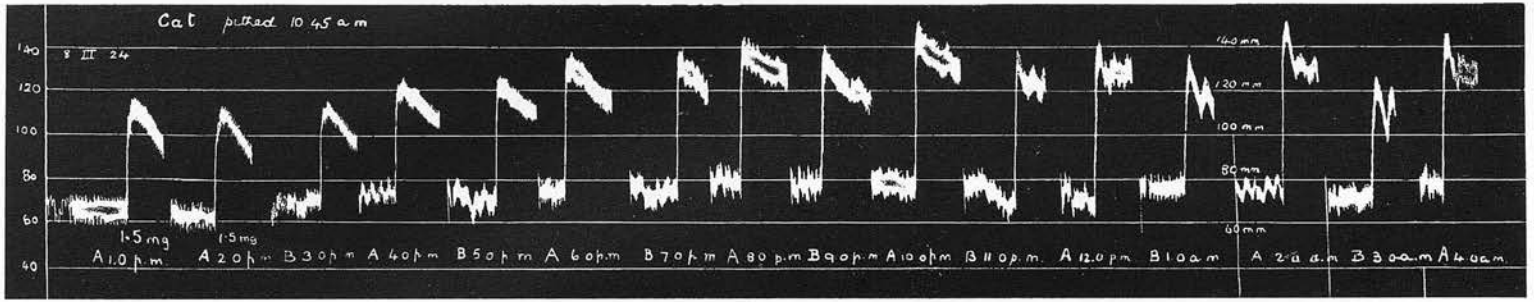

FIG. 6.—Spinal cat. Sixteen successive injections of pituitary extract at hourly intervals commencing 1.0 p.m.: operation at 10.45 a.m. After two initial injections of A, alternate doses of B (which represents a dilution equivalent to 85 per cent., *i.e.* 15 per cent. less than A) and of the standard dose A were administered for the remainder of the experiment. Rate of drum same as in foregoing illustrations, *i.e.* about 2 mm. per second.

activity on a response of the same order and character as the rise produced by initial injection. And if the time required for standardisation by this procedure renders it less accessible to the requirements of com-

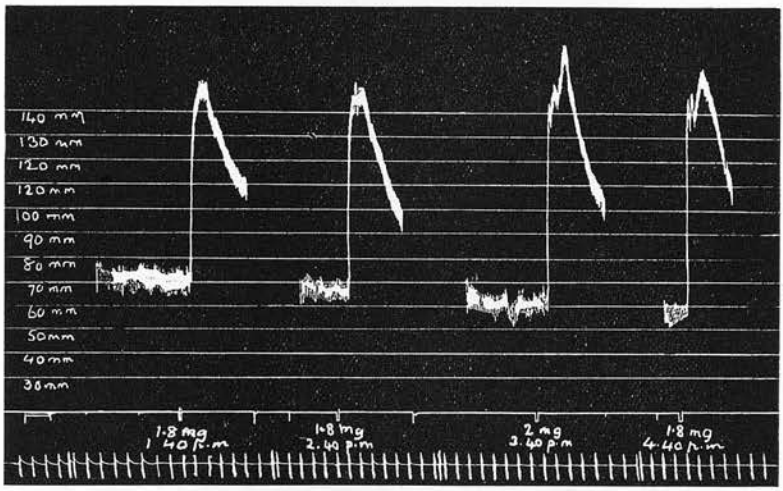

FIG. 7.—Showing 10 per cent. discrimination.

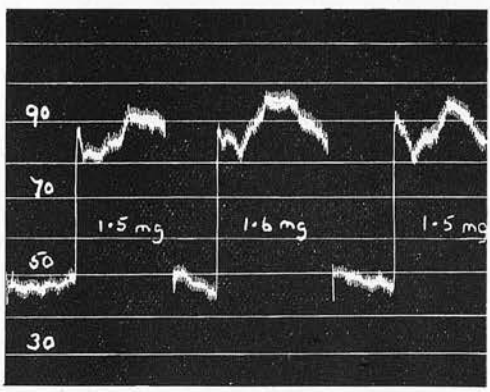

FIG. 8.—Showing 7 per cent. discrimination after thirteen previous injections at hourly intervals. Sixteen hours after operation.

mercial routine than the uterus test of DALE and LAIDLAW, it would appear to be capable of being made as exact as that method.

While not convinced that the pressor principle (*i.e.* the substance producing the prolonged rise on initial injection) is different from the oxytocic component, we think it may be desirable to explore the possibilities here suggested for standardising preparations to be given in

cases of surgical shock ; and we may take this opportunity of pointing out a consideration of clinical importance, especially in the light of DALE'S work on histamine shock. Not only do all commercial preparations display some measure of depressor activity, but one finds samples which give a depressor action on initial injection. Such extracts, if administered to a patient in a state of shock, could only serve to aggravate the evil which their aid is intended to alleviate. This would not arise if the depressor action were destroyed, as suggested earlier, in the manufacture.

4. A COMMENT ON DUDLEY'S RECENT CRITICISM OF ABEL'S WORK.

In connexion with the depressor effect another point deserves attention. The objection has been stated (BURN and DALE, 1922) that "it is not inconceivable that more than one substance may contribute to the total pressor effect. DUDLEY'S recent work affords strong evidence in favour of this supposition, and suggests that tolerance is rapidly established for the most potent of the pressor principles, leaving a remnant of more constant response to a less active principle to which the tolerance acquired is relatively trivial."

By precipitation with sodium picrate of an aqueous extract of the butyl-alcoholic preparation described in an earlier paper, DUDLEY (1923) obtained a crystalline compound to which the pressor-oxytocic substance or substances were adsorbed in high concentration. By extraction with acetone an acetone-soluble fraction R was separated from an acetone-insoluble fraction A. To avoid misunderstanding we may here quote the author's own words: "It is well known that the first dose of a pituitary extract causes a considerably larger response than those which follow ; but after the first few the rise of blood-pressure due to successive equal doses remains fairly constant, if the injections are made at equal time intervals. When A was tested on the blood-pressure the first dose of 0·01 mg. gave a marked pressor effect, but a tolerance was quickly developed so that successive doses produced diminishing responses, until finally 0·01 mg. had very little effect. In no case does the injection of A give rise to the slightest preliminary depressor action. Now doses of 0·5 mg. R showed a strong depressor effect followed by a moderate pressor response. A slight tolerance was developed to R, but strikingly less than the case of A. It was quite impossible to match A and R, and there can be little doubt that we are dealing here with two distinct substances influencing the blood pressure."

To the last sentence it is impossible to take exception ; but we venture to submit that since DUDLEY states that his tests were carried out on the spinal cat, there is no evidence to show that the acetone-soluble substance R of DUDLEY is other than the alcohol-soluble depressor

substance of SCHAFER and VINCENT which, as our observations appear to indicate, exerts a depressor-pressor action on the spinal cat. And since alcohol-extracted pituitary preparations behave precisely like DUDLEY'S substance A, the conclusion to be drawn from DUDLEY'S experiments would seem to be that stated earlier in this paper, namely, the desirability of removing the depressor substance in pituitary extract before attempting to formulate a rational method of pressor standardisation.

It is here perhaps legitimate to refer to certain criticisms directed by DUDLEY against ABEL'S interpretation of the properties of the highly concentrated preparations which have been made in his laboratory. DUDLEY'S actual statement is as follows: "Nothing, however, short of isolation of one pure substance having all these different physiological properties could be accepted as proof of identity" (of the pressor and oxytocic substances). "On the other hand, no absolute or even approximate purification is necessary for disproof of identity: if by any methods the different types of activity can be separated into different fractions even of impure material, we have evidence for the multiplicity of the active principles against which no evidence of failure to separate by any other methods has any weight."

Actual proof of the identity of the uterine and pressor principles admittedly cannot be obtained without isolation of a single pure substance with the properties of the original extract. But unless fractions with different properties can be conclusively shown to be separable, it is correct to preserve the economy of hypothesis to the utmost limit compatible with existing information and attribute the diverse responses evoked by pituitary extracts to the same active substance. Has then, it may be asked, satisfactory evidence of such a separation been put forward? DUDLEY advances the following contention: "In 1919 I described a method by which a partial separation of the pressor and oxytocic principles could be effected. It consists in the extraction of the aqueous solution with butyl alcohol. ABEL and ROUILLER have apparently overlooked this evidence which is fatal to their assumption of identity. I have shown in the foregoing paper that butyl alcohol extraction of a preparation of the same order of activity as theirs removed the oxytocic principle at a much greater rate than the pressor, which remains for the most part behind in the aqueous layer."

While not disposed, for reasons stated elsewhere, to regard ABEL'S preparation as a pure and homogeneous substance, we venture to submit that the truth of this proposition rests entirely on the validity of a single premise, that there exists an adequate method of comparing the pressor activity of pituitary extracts within definable limits. When one speaks of the specific pressor activity of pituitary extract, one presumably refers to the prolonged rise produced on initial injection, apparently DUDLEY'S substance A. Whether or not DUDLEY'S

substance R is different from the depressor substance of SCHAFER and VINCENT, the recognition of this substance in DUDLEY'S preparations is the most cogent criticism of the adequacy of the method which DUDLEY himself has employed to compare the pressor activity of different samples. That we are not alone in drawing this inference is suggested by the citation taken from BURN and DALE'S memoir. Though tentative experiments of our own tend to confirm the view that the pressor and oxytocic substances are not identical, we cannot regard DUDLEY'S conclusions as fully established, until his fractions have been standardised by a method of comparison such as we here propose, based upon the comparison of successive responses of *the same character and the same order of magnitude* as the rise evoked by initial injection.

5. SUMMARY.

1. A method of standardising pressor activity of pituitary extracts with an order of precision not significantly inferior to that obtained with the virgin uterus of the guinea-pig is described.

2. For quantitative or clinical purposes involving action on the circulatory system, pituitary extracts free of depressor substances can be prepared, as SCHAFER and VINCENT indicated, by alcoholic extraction.

3. For depressor-free extracts tolerance is a function of the dosage and time interval between successive injections.

4. With appropriate time intervals a remarkable constancy of response of the same character and order of magnitude as the initial rise in blood-pressure may be obtained with the spinal cat for periods up to twenty hours.

5. With submaximal doses the period of recovery for response of the same order as that obtained on initial injection is rather less than an hour.

6. Consistent discrimination tending to increase as the experiment proceeds may be obtained for ten or twelve hours between hourly doses differing by 10 per cent.

7. The curve obtained by plotting increase of pressure against dose shows the steepest gradient near a point corresponding to half the dose requisite to produce maximal response ; and the best discrimination is obtained by working with a standard in the neighbourhood of this value.

8. It is therefore suggested that the match should be made against a standard for which a curve of reference is kept in use : the limit of accuracy can be defined in any instance by interpolating a known dilution between two injections of the standard.

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(For other papers quoted consult previous contributions of this series.)

THE ACTION OF PITUITARY EXTRACTS ON INTESTINAL MUSCLE.

By A. D. MACDONALD. From the Department of Physiology, University of Edinburgh. (With six figures in the text.)

(*Received for publication 9th February* 1925).

INTRODUCTION.

THE divergent findings of the numerous investigators of the action of extracts of the pituitary body on intestinal muscle is not surprising in the light of the differences involved in the materials and techniques employed. YOUNG (20), using isolated muscle preparations, found that the movements were much increased by aqueous extracts of posterior lobe in the case of the cat, but inconstant for the rabbit. He found it necessary to use concentrations of 0·04–0·1 per cent. (2–10 glands in 100 c.c. of his Ringer) to elicit a response. BLAIR BELL (4) refers to "a remarkable pressor action on intestinal muscle," intravenous injection of his extracts regularly producing defæcation in the pithed rabbit. His findings on the isolated gut, however, were "relaxation and cessation of the normal movements, but often preceded by powerful contractions." Stronger extracts than Young's were used (2–10 per cent.), whereas ATWELL and MARINUS (2) report inhibition with 0·0002 per cent. BAYER and PETER (3) found as a typical response (to 10–15 per cent. of the "pituitrin" of Parke, Davis) inhibition, sometimes followed by increased activity. SHAMOFF (17) actually compares his findings with pituitary to those obtainable with adrenaline, inhibition being marked with concentrations of 0·0002–0·001 per cent., whether using his own laboratory products or various reputable proprietary preparations. On the other hand, O'CONNOR (21), commenting on the fact that the inhibition of the movements of the gut by adrenaline can be antagonised by the addition to the fluid of an amount of serum equivalent to the concentration of adrenaline, considers that this is due to the serum containing a stimulant, and "in all likelihood it may prove to be infundibulin." DEGENER (8) maintains that the response to pars nervosa is relaxation, opposite to that given by the glandular part (contraction), but that if the animal be fed on oatmeal and milk both lobes give the latter effect. UNO (18) agrees with BLAIR BELL, that "hypophyseal extract causes a relaxation of the slip, preceded by a slight short contraction," but finds that when the animal is killed after

severe exhaustion, as by fighting, its hypophysis yields a stimulating extract. DALE and LAIDLAW (7) found no increase in the intestinal movements in the dog following intravenous injection of the extract, but attribute this to the intestinal anæmia produced by the contractions of the vessels. DIXON (9) finds that intravenous injection of commercial extracts produces increase of tone, but not of peristalsis, in the small intestine, but not in the large. DALE'S observations on the isolated gut, however, enable him to conclude that "the characteristic action of extracts of the posterior lobe is stimulation of plain muscle fibres" (6), a view upheld by all the textbooks the writer has examined.

EXPERIMENTS.

A large number of pituitary preparations, both commercial and laboratory, have been tested. It is felt that little importance can be attached to commercial preparations, since the means of production, dissection, extraction, sterilisation, or preservation are either not divulged or may be open to criticism. Laboratory products similar to those described in a recent joint paper (13) are not open to such objections. Glands have been removed from the freshly killed animal and extracted with the Ringer used for perfusing the muscle strip. Other glands have been rapidly dried and ground and the extracts prepared as required, again using the appropriate Ringer. Ox glands have been obtained immediately after killing, rapidly dehydrated in acetone, transferred to ether, and the lobes then separated, minced, and dried in an oven at 60°–70° C. The resulting product is finely ground, sifted through muslin, and extracted with absolute alcohol in a Soxhlet apparatus for at least six hours, to remove the "depressor" component (see 12 and 13). The result is a powder of which the extracts compare very favourably with all other pituitary preparations available if compared by the uterus or blood-pressure tests. In addition to these extracts, the residues left on evaporating to dryness, the acetone, ether, and alcohol used in the preparation, have been tested. The experiments quoted have been performed on slips of ileum (terminal portion) from the cat, using SHARPEY-SCHAFER'S modification of the Magnus method (16). Slips of longitudinal coat about 2 cm. long and 0·5 cm. broad were employed, stripped off as thinly as possible. The Ringer recommended by DALE for use in the uterus bath, omitting the glucose, was found suitable. It has the following composition :—

NaCl	90·0 grams.
KCl	4·2 ,,
$CaCl_2$	2·4 ,,
$NaHCO_3$	5·0 ,,
$MgCl_2$	0·05 ,,

Distilled water to 10 litres.

The ideal working temperature for slips in this Ringer is 34°–35° C., and because of great sensitivity to small variations in temperature (as indeed also to changes in pH or osmotic pressure), this was rigidly maintained. A few observations made on gut from the dog, rabbit, and guinea-pig seemed to be in agreement with these made on the cat.

While the "infundin" of Burroughs Wellcome never failed, if given in adequate concentration, to show a stimulating effect, the "pituitrin" of Parke Davis often seemed to be entirely without this principle even when used in much higher concentration. With the

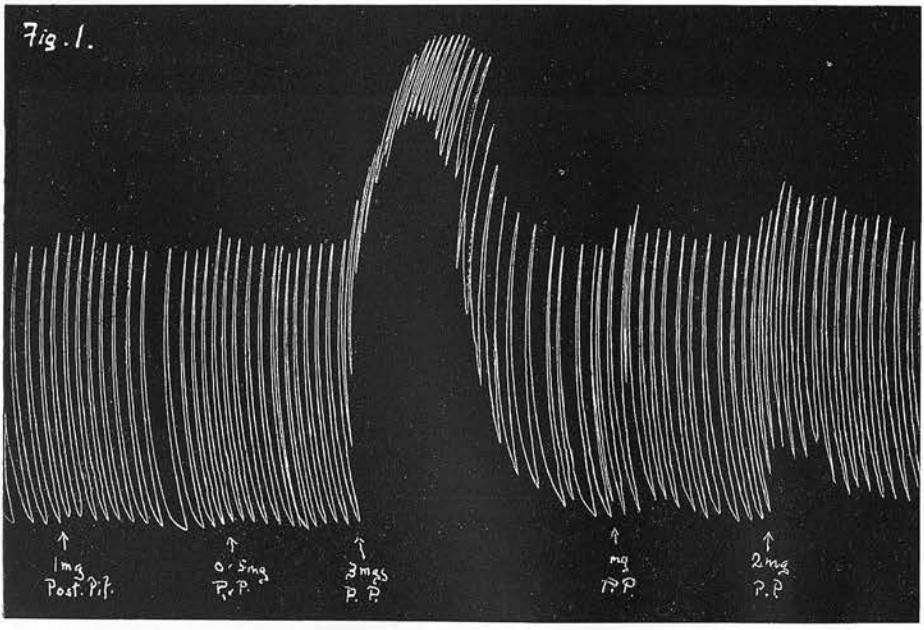

FIG. 1.—Showing the response of a slip of ileum to saline extracts of 0·5, 1, 2, and 3 mg. desiccated pituitary gland, posterior lobe. With the last only is there a marked effect.

laboratory preparation of ox glands (fig. 1) a marked stimulation is not obtained with less than 3 mg., representing a transitory concentration in the Magnus tube of not more than 0·2 per cent. Smaller doses than 0·5 mg. were entirely without effect, and the writer failed absolutely to get the inhibition claimed by previous writers. With anterior (glandular) lobe extracts even in much higher concentrations no effect whatever has been obtained.

There is much evidence in work done in this laboratory (12) and elsewhere (ABEL and KUBOTA (1)) for the presence in most posterior lobe preparations of a histamine-like substance, the "depressor" substance. To histamine stimulation the preparations of intestine used are amazingly sensitive. Thus in fig. 2 there is a well-marked effect

for a dose of ergamine acid phosphate, 0·0002 mg. (corresponding to histamine, 0·00007 mg.). This would indicate that histamine has some 40,000 times the available stimulating power of dried gland. Now, histamine has only some 400 times the oxytocic power of the gland, computed from the observations of BURN and DALE (5). While the dose of dried gland for the maximum effect on the blood-pressure of the cat is less than 3 mg., histamine does not give a marked effect with doses of less than 0·01 mg. Thus, while enough of the "depressor" principle has been removed to abolish a blood-pressure response, there may yet be enough to stimulate the intestine; and since the active dose of this principle is so minute it is not difficult to appreciate the probable impossibility of removing all the stimulant from the dried gland.

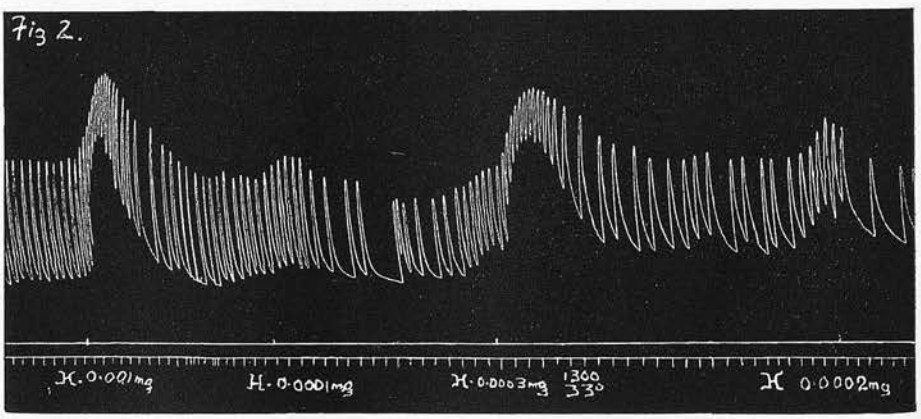

FIG. 2.—Showing the response of a slip of ileum to small doses of histamine; 0·0002 mg. of "ergamine acid phosphate" gives increased tone and rate. Time tracings throughout show minutes.

In fig. 3 is shown a comparison of the responses of a slip of ileum to saline extracts of—

(1) P.P., 5 mg. desiccated posterior lobe hypophysis, previously extracted with ether and absolute alcohol.
(2) E.E., 1 mg. of the dried residue of an ether extraction of gland in a Soxhlet apparatus (six hours).
(3) A.E., 0·2 mg. of the dried residue from an extraction (Soxhlet, six hours) with absolute alcohol.

It is to be noted that (2) E.E. is practically free from both pressor and oxytocic principles, while (3) A.E. contains only the "depressor" principle, if tested on an etherised cat. As to whether or not some part of these responses may be attributable to lipoid content cannot be dealt with at present, but it is clear that the alcohol residue contains more than five times the stimulant of the ether residue and more than twenty-

five times that of the extracted desiccated gland, though this last is still very rich in both pressor and oxytocic principles. YOUNG (20) found that only " a slight rise of tone was obtained by alcoholic extraction, probably due to the want of dehydration," and reports in his summary " Alcoholic extract has no action." Clearly his extraction, rather than his dehydration, was incomplete, since his glands were taken from chloroform, finely minced, and dried in an incubator for twenty-four hours at 40° C.

It seems generally to have been assumed that the intestinal stimulant of the pituitary is without parallel in most other tissues, but this is not the case. In fig. 4 is shown a series of responses to extracts from

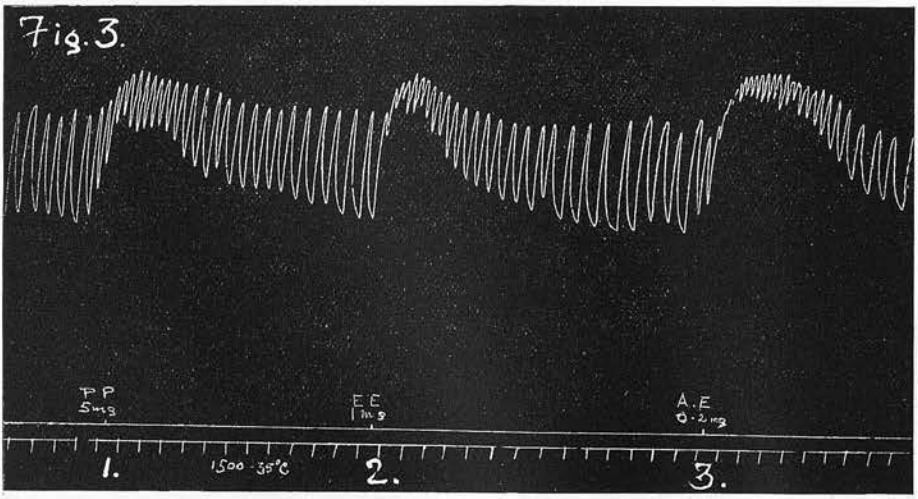

FIG. 3.—Showing the response of a slip of ileum to saline extracts of gland substance, and the residues obtained on evaporating ether and alcohol extracts of the same.

tissues removed from a freshly killed guinea-pig. These were weighed, minced, and extracted with boiling saline to give equal concentrations (0·25 per cent.). From the tracing it is evident that the hypophyseal extract is poorer in stimulant than the neighbouring piece of brain. Desiccated tissues were also prepared, and in fig. 5 one may compare the relative activities of ground, dried posterior lobe gland (Q) and heart-muscle (H). While the H effect is less than that of Q, the order of difference seems negligible, so that one cannot support any pretension to specificity in this " remarkable pressor principle of the pituitary gland."

It has been established that the pressor and oxytocic principles of the hypophysis are rapidly destroyed by boiling with alkali. GUGGENHEIM (10) has brought forward evidence that the pituitary stimulant of intestinal muscle is not so destroyed. In the light of the foregoing this is not surprising, since it does not seem to be either of these established

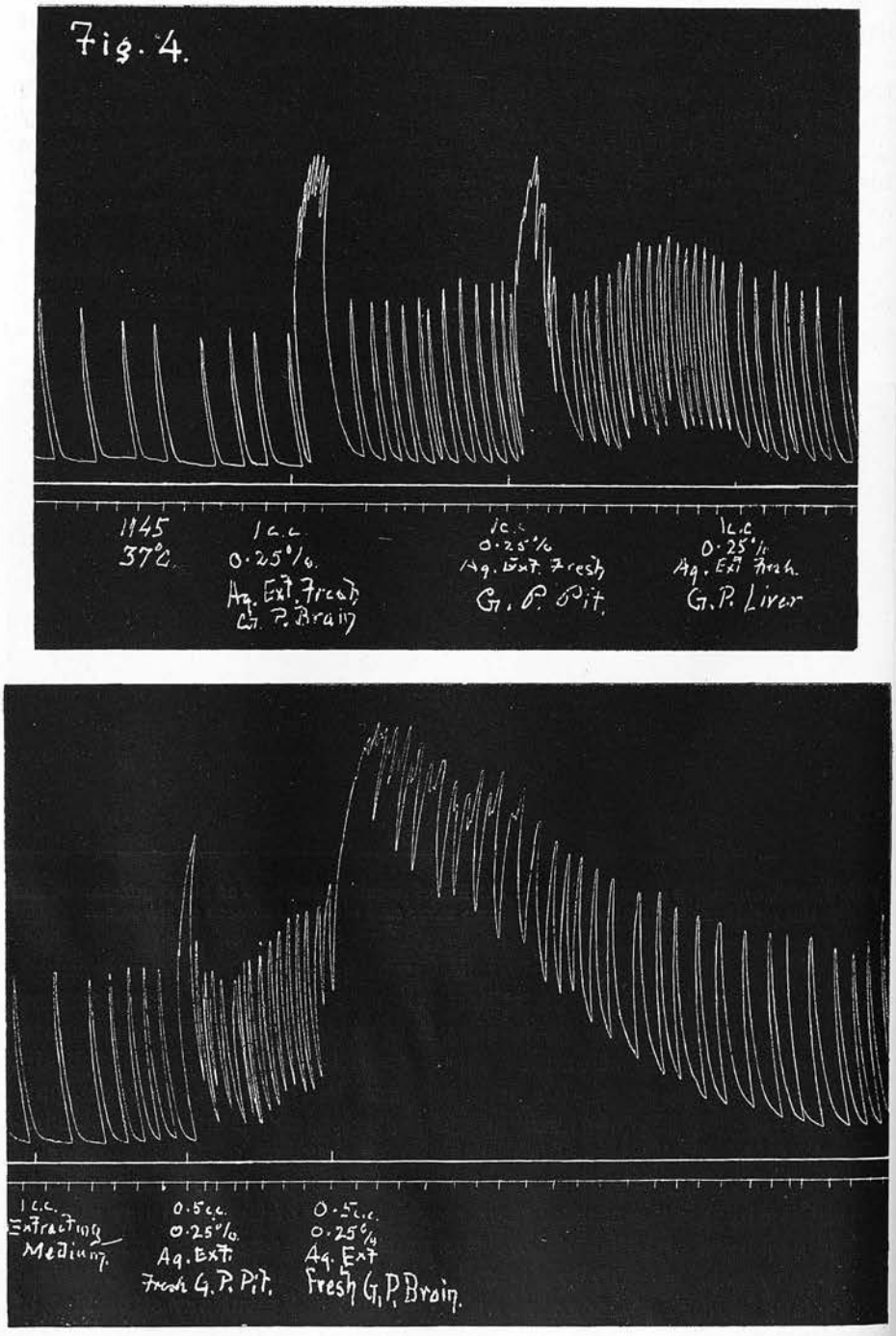

FIG. 4.—Showing the absence of specificity in the response to pituitary extracts. A greater effect is produced here by an extract of fresh brain than by an extract of freshly dissected hypophysis, in the same dilution.

principles which is responsible for such stimulation. Fig. 3 indicates

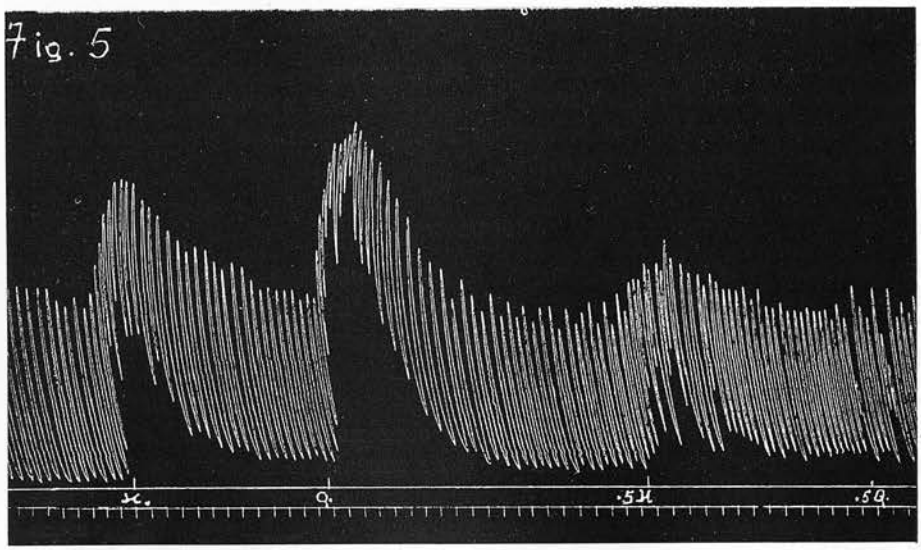

FIG. 5.—As fig. 3, but comparing extracts of desiccated gland and heart muscle.

Q=1 c.c. 0·25 per cent. saline extract desiccated posterior lobe pituitary.
H=1 c.c. 0·25 per cent. saline extract desiccated heart muscle (guinea-pig).

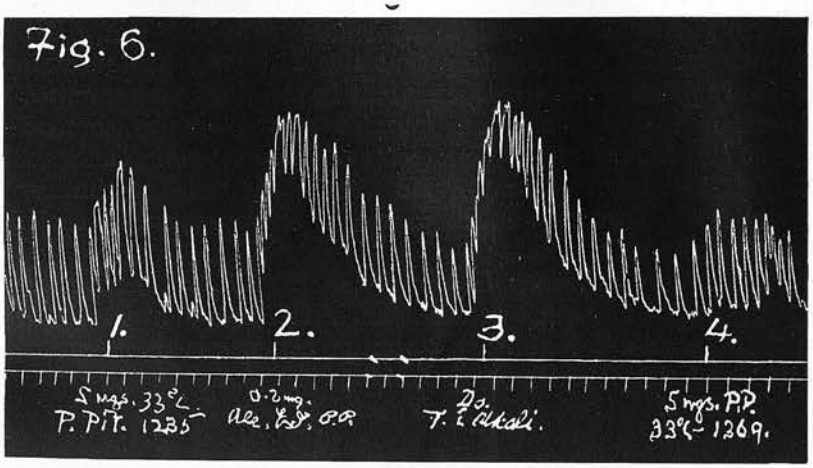

FIG. 6.—Showing the response to 5 mg. gland and to 0·2 mg. of the residue obtained from a Soxhlet extract with absolute alcohol (six hours) before and after alkali digestion.

that the intestinal stimulant can be greatly concentrated by alcoholic extraction. In fig. 6 is seen the responses of a slip of muscle to—

(1) 5 mg. desiccated posterior lobe hypophysis.
(2) 0·2 mg. residue on alcoholic extraction (A.E. of fig. 3).

(3) The same, boiled for thirty minutes with decinormal NaOH and neutralised before application.

(4) 5 mg. desiccated posterior lobe hypophysis.

(2) and (3) are of the same order of magnitude, hence the intestinal stimulant not only differs from the pressor and oxytocic principles in being alcohol-soluble, but is not destroyed by chemical processes which these principles cannot withstand.

Discussion.

Perhaps it is not surprising to find that commercial extracts should fail to give consistent effects on such an indicator as the isolated gut. So far as the posterior lobe of the pituitary is concerned, it is not only the case that there are great differences between the products of different firms, but different batches of the same preparation show considerable variation in their content of intestinal stimulant, *e.g.* " pituitrin " may be free from it or contain it in very considerable amount. Even in laboratory products prepared with scrupulous care under identical conditions differences arise. The intestinal stimulant certainly seems to show a remarkable similarity to the depressor principle, a histamine-like substance which may apparently also be present in variable amount and from which perfectly fresh preparations may be free.

Some observations made in another connexion may throw light on those discrepancies, as indicating that this histamine-like substance is not the only possible intestinal stimulant in pituitary preparations. In collaboration with Hogben an attempt was made to confirm the statement (11) that pituitary extracts have a specific action on the heart of the cold-blooded vertebrate. No account of the experiments has been published elsewhere, and the present is an appropriate occasion for summarising the main points :—

(1) Laboratory depressor-free products, even in a concentration of 10 per cent., have no action on the heart of the frog and tortoise.

(2) The invariable inhibitory action of commercial preparations on the same material depends on the acidity of the medium in which the drug is distributed (1 c.c. of a commercial extract X may increase the acidity of 100 c.c. of Clarke's Ringer from pH 8·4 to pH 6·4).

(3) A definite inhibitory effect in alkaline media was obtained on the heart of the dog-fish in such dilutions as 1 in 1000. The writer has shown that this heart is exceedingly sensitive to parasympathomimetic drugs (15). The intestine is also sensitive to such ; *e.g.* 1 in 50,000 choline increases its tone and movements. Histamine has very little action on the isolated heart of the cold-blooded vertebrate, therefore the possibility of the occurrence of choline-like substances, even in laboratory glandular extracts, must not be overlooked.

The work described in this paper makes it clear—

(1) that pituitary extracts vary greatly in their content of intestinal stimulant.

(2) That the intestinal stimulant differs from the other known pituitary principles in being alcohol-soluble and alkali-stable.

(3) That extracts of laboratory preparations do not act in concentrations to which much physiological significance can be attached. A muscle-slip just sensitive to 1 in 1000 pituitary responds to adrenaline in a concentration of 1 in many millions, while the same pituitary will stimulate the isolated uterus of the virgin guinea-pig in a dilution of 1 in 500,000.

(4) That the pituitary is not materially richer in the stimulant than several other tissues.

Since pituitary extracts have been shown by WADDELL (19) to fail to stimulate the frog's œsophagus, and by HOGBEN and HOBSON (14) to be without action on such invertebrate plain muscles as the crop of Aplysia and the pharynx of Aphrodite, it seems that the conception of such extracts as a general excitant of plain muscle requires modification.

Extracts of posterior lobe have acquired some clinical reputation in the treatment of obstipation, and cases come to light from time to time in which such therapy has succeeded after all else has failed. It seems difficult to believe that after any ordinary dosage the principle could reach the gut itself in active concentration. Where an attempt to remedy constipation in a hospital ward by means of pituitary has been made, the results have not been at all convincing (22). BLAIR BELL's observation, that injection of pituitary extract regularly produced defæcation in the pithed rabbit, does not seem to apply to other animals. In over one hundred cats, anæsthetised or pithed, subjected to injections of the specially prepared laboratory preparations of the gland used in the work of HOGBEN and SCHLAPP (12), and HOGBEN, SCHLAPP, and MACDONALD (13), defæcation only occurred twice, although some of the experiments were continued with hourly injections for the whole day.

SUMMARY.

Doubt is thrown on the commonly held view that the pituitary gland contains a general stimulant of plain muscle fibres, since for the isolated intestine there is no evidence of action in such concentration nor of such tissue specificity as would justify that belief.

The expenses of this research were defrayed by a grant from the Earl of Moray Fund of the University of Edinburgh.

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THE ACTION OF EXTRACTS OF THE POSTERIOR LOBE OF THE PITUITARY BODY ON THE PULMONARY CIRCULATION.

By E. SHARPEY-SCHAFER and A. D. MACDONALD. From the Department of Physiology, University of Edinburgh. (With twenty-eight figures.[1])

(*Received for publication* 14*th May* 1926.)

THE experiments on which the following observations are based are a continuation of work begun some years ago in this laboratory by R. J. S. M'DOWALL (1), which formed the subject of his thesis for the D.Sc. degree in 1921. As the work was in many respects incomplete it was not published ; but the thesis is preserved in the Edinburgh University Library, and can be consulted there by anyone who may desire to do so. Unless otherwise specifically mentioned, any references we may make to M'DOWALL'S work must be understood to refer to that contained in the thesis.

The following is a brief abstract of the thesis :—

After a short historical introduction in which reference is made to the views which obtained regarding the pulmonary circulation up to the time of HARVEY, the author proceeds to describe the results of modern methods which have been used for the investigation of the flow of blood through the lungs in the living animal—beginning with those of LUDWIG and BEUTNER in 1850, and ending with those of SHARPEY-SCHAFER and LIM in 1919. He then proceeds to give a description of his own experiments on the effect of extracts of the posterior lobe of the pituitary on the pulmonary vessels—commencing with the results of perfusion experiments on the vessels of the surviving lung, and proceeding to relate the effects produced in the intact anæsthetised animal by intravascular injection of the extract in question.

M'DOWALL found that the perfusion effect of a first dose was generally to cause constriction lasting a long time ; subsequent doses always caused a short-lived constriction followed by prolonged dilatation, but this was also often got with a first dose. Exceptionally the dilatation failed to show itself with a second and subsequent doses, especially in the rabbit.

M'DOWALL'S results on the anæsthetised animal by intravascular

[1] The tracings illustrating this paper are for the most part considerably reduced for reproduction.

injection varied greatly. In some experiments the first dose produced relatively an even larger rise in the pulmonary than in the aortic system. In others, when the aortic pressure rose the pulmonary fell. Subsequent doses produced a rise in the pulmonary and a fall in the aortic system. M'DOWALL found no constant difference between the cat and the rabbit.

Besides the principal subject, the thesis includes an account of spontaneous rhythmic contractions observed in surviving perfused blood-vessels belonging both to the aortic and to the pulmonary system. The history of this subject is given and his own results are recorded. The chief fact bearing upon our work is that large waves occur frequently in perfusion of both systemic and pulmonary vessels and also in the anæsthetised animal. In the latter they may be seen in one of the systems and not in the other, indicating that they are not the result of rhythmic activity of the vasomotor centre. Further, they are sometimes produced after an injection of pituitary extract when they may not have been showing before.

That the thesis represents a large amount of work may be gathered from the fact that it is illustrated by no less than forty-nine kymographic records, each accompanied by a description of the procedure adopted.

The only other observers who appear to have given special attention to the action of post-pituitary extract upon the pulmonary circulation are WIGGERS and HALLION. WIGGERS (2) described a lowering of pulmonary pressure as the effect of intravenous administration of the extract, but most of his experiments were directed to ascertain its action on the individual heart-beats. HALLION (3), in a very short paper, illustrated by a single tracing, gives the result of an experiment upon the dog, in which he obtained a prolonged fall in the pulmonary pressure and a corresponding prolonged rise in the aortic : the latter preceded by a short preliminary fall. He also records a rise of pressure in a pulmonary vein, corresponding with the fall in the pulmonary artery. He mentions that in other experiments he obtained only a slight rise in aortic pressure, often with large oscillations ; but he always got a distinct fall in pulmonary pressure. Without giving reasons for the opinion, he is inclined to ascribe this fall to a failure of the right side of the heart—*un ralentissement de débit du cœur droit.*

Most of M'DOWALL's experiments on living animals were made by the method described by one of us (4), and as it is the method we have used in this research, the results are—as far as they go—comparable. The chief difference in our experiments as compared with those of the previous observers is that we have separated from the pituitary posterior lobe the two substances which it generally contains, which have entirely different effects on the circulation. They can be separated by extraction with absolute alcohol (5), which removes a substance producing a depressor action on the systemic circulation and a pressor action

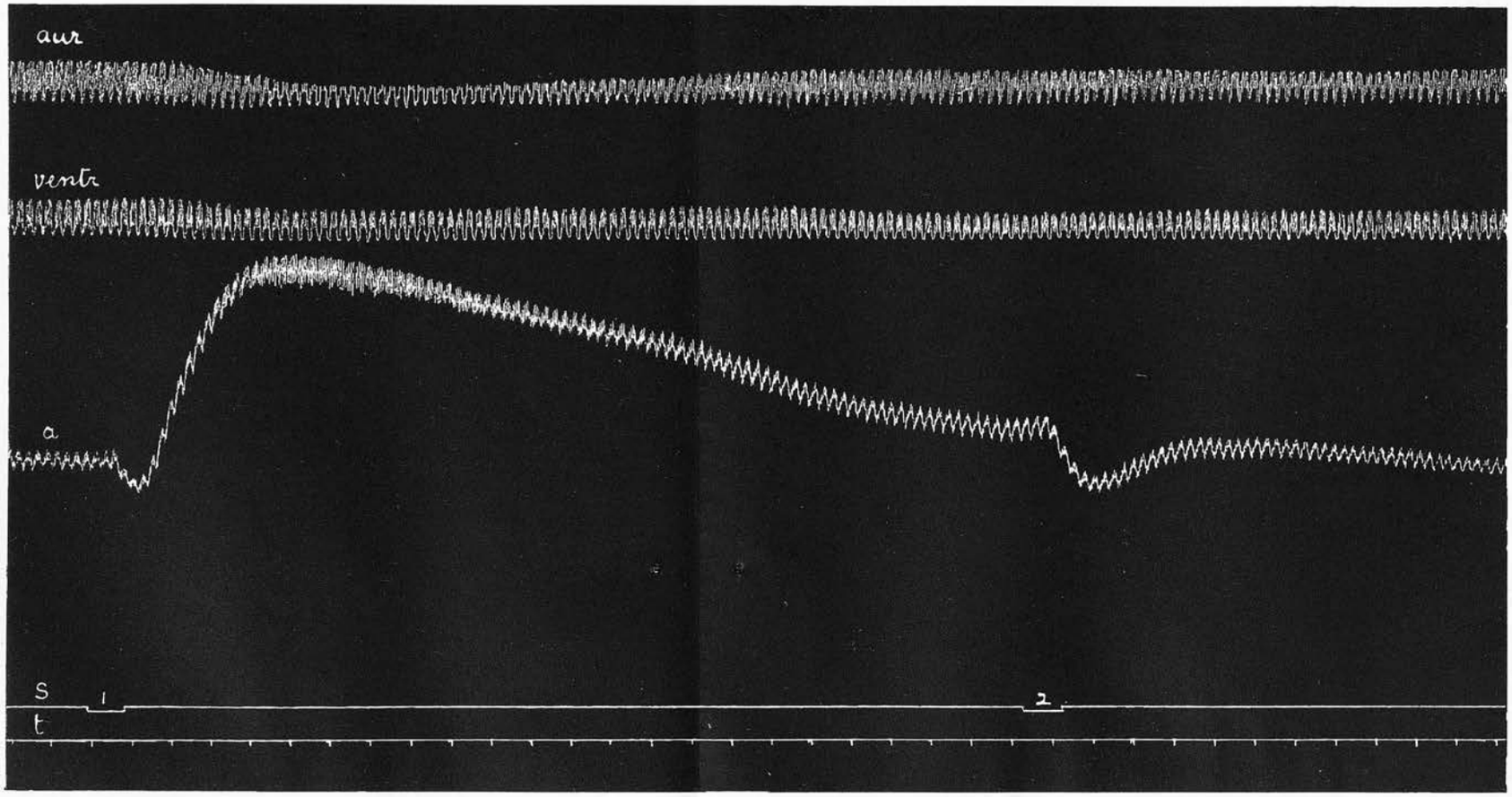


FIG. 1.—Cat, chloralose. Effects of a first and second dose of an extract of bovine posterior lobe, not previously extracted with alcohol.

aur, tracing of right auricle; *ventr*, tracing of right ventricle; *a*, aortic pressure; *s*, signal; *t*, time in 10″ intervals. The pulmonary pressure was not recorded in this experiment.

1. Effect produced by intravenous injection of 1 c.c. of Ringer, representing 5 mgm. of gland.
2. Effect of a second dose of the same extract administered four minutes after the first.

The effect of the first dose is to cause a preliminary fall of aortic pressure followed by a rapid rise, and this by a gradual descent towards the original level. Notice the increased extent of the fluctuations in pressure near the summit of the rise. These are due not to an increased force of contraction but to a slowing of the rhythm, as may be seen by a glance at the cardiac records. Notice also the weakened cardiac contractions: the effect is less on the ventricle than on the auricle, but is quite evident in both.

The effect of the second dose produces only a fall of aortic pressure like that caused by a small dose of histamine. Complete immunity is established for the "pressor" effect on aortic pressure and also for the cardiac slowing and weakening.

SHARPEY-SCHAFER and MACDONALD, "The Action of Extracts of the Posterior Lobe of the Pituitary Body on the Pulmonary Circulation."

on the pulmonary system. The residue, after extraction with alcohol, acts upon the circulation, both pulmonary and aortic, in a manner quite different from the alcohol-extract, as will be described later. Further, it exhibits the remarkable property of producing, when given in adequate dose, complete or partial immunity to subsequent doses of the same substance administered within a certain time, varying according to the original dose. The substance extracted by alcohol, which appears to be histamine, does not show this phenomenon. Hence it happens that if an extract containing both these substances is employed (fig. 1), a first dose will usually produce a rise in systemic pressure, which is sometimes preceded by a fall; whereas a second and subsequent doses administered intravenously within a certain time will cause only a fall, either without any rise at all or with a slight and deferred rise (5). A similar deferred rise is obtained with histamine, and may, as BURN and DALE (6) have suggested, be produced by excitation of secretion of adrenaline.

We have used in our experiments (*a*) solutions or decoctions made from dry pulverised posterior lobe which has been extracted with Ringer's solution alone, and have compared the results with (*b*) those of solutions or decoctions prepared from alcohol-extracts of such dry pulverised posterior lobe, as well as with (*c*) solutions of histamine (ergamine acid phosphate), and with (*d*) water-extracts of dry pulverised posterior lobe which had been thoroughly extracted with alcohol and which therefore contained no histamine. We have further used as a comparison commercial extracts, which invariably contain both substances, and usually also some special preservative material which may or may not have a physiological action: these commercial preparations may, broadly speaking, be included in the class (*a*) referred to above. As already remarked, the previous workers on the effect of pituitary on pulmonary pressure have only made use of extracts containing both constituents. And it may be stated generally that, with one or two exceptions, all the earlier work on the effect of extracts of the pituitary on various organs of the body has been complicated by the failure to recognise that the extracts used have contained two differently acting substances, and as the proportion of each of these may vary in different extracts, no constant results were to be expected. Since the alcohol-soluble substance in the extracts has been shown to be histamine (7 and 8), which can be entirely removed by prolonged extraction with alcohol, attention must be directed in future investigations to the relative parts played by this substance and by the other active principles present in the residue after its removal. These we have treated as a single entity under the title of histamine-free substance. This has been necessary, because we know no means by which the residue after alcoholic extraction can be adequately separated into definite components which differ in their physiological action. But as there is some reason to

believe that the pressor, oxytocic, melanophore, and other effects which are obtained are due to specific autacoids (9), it is highly probable that the histamine-free residue contains not one but several such autacoids, and not surprising if different samples are not found always to produce exactly the same effects. However this may be, the general results obtained from the action of decoctions of the " residue " are sufficiently constant to enable one to form some idea of its action on the circulatory system.

The variation in the amount of histamine in commercial pituitary preparations is certainly sufficient to account for the considerable variation met with in their effects. The question of the administration of pituitary extract with or without histamine is therefore of therapeutic importance. Histamine is a powerful drug possessing distinct and probably valuable physiological properties. But it is contra-indicated in situations in which the " residue " of the posterior lobe extract is valuable. Although it has an oxytocic effect on the uterus of some animals (*e.g.* guinea-pig), this effect is not nearly as strong as that of the histamine-free " residue " (10) ; in other animals (*e.g.* rat) histamine has no effect on the uterus, although this organ is powerfully acted upon by pituitary " residue." Moreover, histamine is a powerful agent in stimulating intestinal muscle, and since commercial pituitary extracts are used for this purpose and intestinal muscle is not stimulated by the histamine-free residue (11), histamine might with advantage be administered independently, if it is only loss of tone of the bowel which is to be combated. On the other hand, the histamine-free residue tends to impart tone to the blood-vessels, including, as KROGH has suggested, the capillaries, whereas DALE and his co-workers (12, 13) have found that histamine causes the capillaries to dilate.[1] And since pituitary extract is used to counteract the effects of " shock "—and this condition is believed to be due, in large measure, to capillary dilatation—the extract would be more efficient if freed from histamine, which itself tends to produce " shock." This consideration has been already urged by HOGBEN, SCHLAPP, and MACDONALD (8).

According to INCHLEY (14), the dilatation of capillaries under the influence of histamine described by DALE is due rather to venous constriction than to a direct dilator effect on capillaries, since histamine has a powerfully constrictor influence on venules and might in this way cause passive dilatation of capillaries. INCHLEY finds that the pulmonary venules are especially sensitive to histamine.

METHODS.

For the purposes of this investigation twenty-seven cats, seventeen dogs, and twenty-one rabbits have been employed, and one monkey.

[1] In carnivores (dog and cat), but not in rodents (rabbit and guinea-pig) ; in these all blood-vessels are contracted by histamine.

Except for preliminary procedures, the use of inhaled anæsthetics, such as chloroform and ether, has been avoided, since these might be liable to produce a directly paralysing effect on the nerves and musculature of the pulmonary vessels. Nevertheless, such experiments as were made with ether as an anæsthetic throughout, revealed much the same results as when chloralose or urethane had been employed. For anæsthetisation during the experiment, administration (intravenous or hypodermic) of chloralose, urethane, or morphia has generally been used. When it was considered desirable to eliminate the effects of anæsthetics altogether, recourse was had to Sherrington's decerebration method. For a few experiments " spinal " animals were used, the cord having been cut at the level of the axis vertebra. When artificial respiration was needed this was given by means of a Brodie pump, driving warmed air intermittently into the lungs. The animals were kept throughout the whole of the experiment on a warmed Brodie operating-table.

The method we have employed for preparing the extracts is the same as that described by W. SCHLAPP (15). The posterior lobes having been separated from perfectly fresh bovine glands obtained at the slaughter-house, the water and fats were partially removed by placing the tissue in a large excess of acetone and subsequently in ether. The material was then minced, dried at 70° C., and finely ground. Part of the powder was extracted with absolute alcohol in Soxhlet's apparatus for twelve hours to remove the histamine. The residue, which was left after the alcohol extraction, was dried, and decoctions made with Ringer's solution representing weighed amounts of the dry residue. This we call *histamine-free substance.* Other decoctions were made from weighed amounts of the dried alcohol-extract ; these contain *histamine.* Yet other extracts were made from the dry unextracted gland ; these, therefore, contained both substances. Small doses (1 c.c), representing the extract of from 1 mgm. (for rabbit) to 10 mgm. (for dog), of dry material were given. The doses were always warmed to the temperature of the body before being administered.

The operative procedures included the implacement of a tracheal cannula for artificial respiration, the insertion of an arterial cannula into the carotid or femoral artery for recording the aortic pressure with a mercurial manometer, the opening of the chest by splitting the sternum, and the insertion of a specially constructed cannula (curved near the extremity for the rabbit) into the pulmonary artery through the ventral wall of the right ventricle. This cannula was connected with a manometer containing half-saturated solution of bicarbonate of soda, which gives a manometric pressure very nearly the same as water ; the fluctuations in pressure are recorded by an Ellis piston-recorder. If it was not desired to register the heart-beats, the chest was sewn up with the lungs inflated, and the artificial respiration, which had been necessary while the thorax was open, was stopped ; the natural respiratory movements

were then resumed, and recorded by tambours. But usually the chest was left open, and an auricle and ventricle, generally the right, were attached by fine hooks and silk thread to the cardiomyograph (fig. 2), which took the same form as that which was used by OLIVER and SCHAFER (16) in their original experiments on the action of suprarenal extract. This, of course, rendered it necessary that artificial respiration should be maintained during the whole experiment. Nevertheless, it

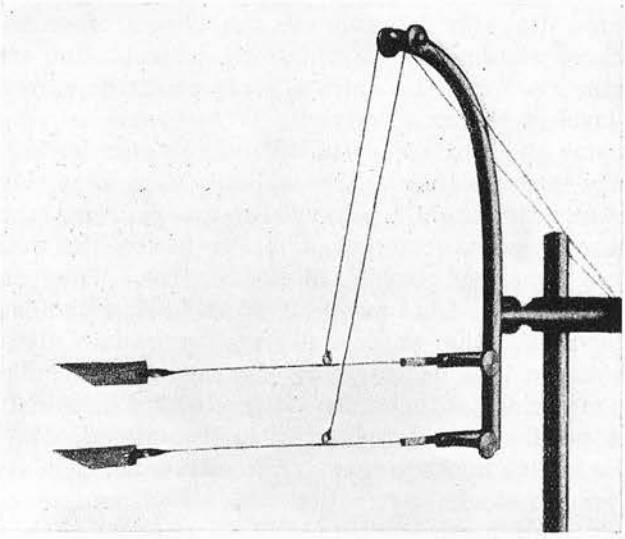


FIG. 2.—Cardiomyograph for recording the rate and extent of contraction of auricle and ventricle of the exposed heart.

often happened that natural movements of respiration continued throughout; when this was the case they were recorded by the double respiratory tambour system; this also sometimes showed the rate of the artificial respiration as well as the natural movements.

RESULTS.

When an extract of the whole post-pituitary substance, containing therefore both principles, is administered in an adequate dose to a chloralosed cat, the effect on the pulmonary pressure is to cause a short rise followed by a slight but more prolonged fall; on the aortic pressure a sharp fall followed by a rapid rise, which again is succeeded by a gradual fall (fig. 3). If a "repeat" dose of the same extract is given within a short time after the first, only the short pulmonary rise and the corresponding aortic fall are seen; the latter may be followed by a slight after-rise (fig. 4). If, now, in the same animal an equivalent dose of post-pituitary substance which has been deprived of histamine by

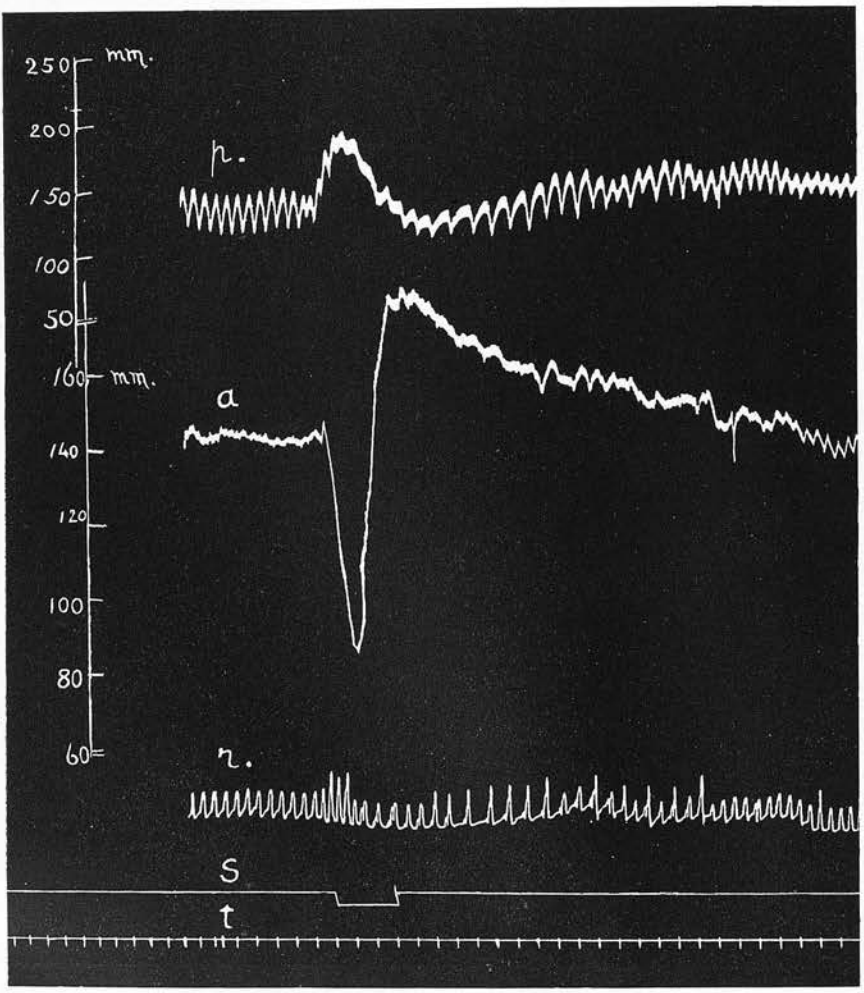


FIG. 3.—Cat, chloralose. Effect on pulmonary and aortic pressures of a first dose of extract equivalent to 5 mgm. of posterior lobe of ox pituitary, not previously extracted by alcohol and containing therefore both substances. The thorax is closed in this and several succeeding experiments, so that the cardiac movements are not recorded. Lettering as in fig. 1, but with the addition of *p*, pulmonary blood-pressure (in mm. H_2O), and *r*, respirations. (In this and all other tracings the respiratory movements recorded are active.) Notice the rise in pulmonary pressure followed by a more prolonged fall, and this by a gradual rise, the sharp fall in aortic pressure (more marked than usual) followed by an equally sharp rise, and this by a gradual fall.

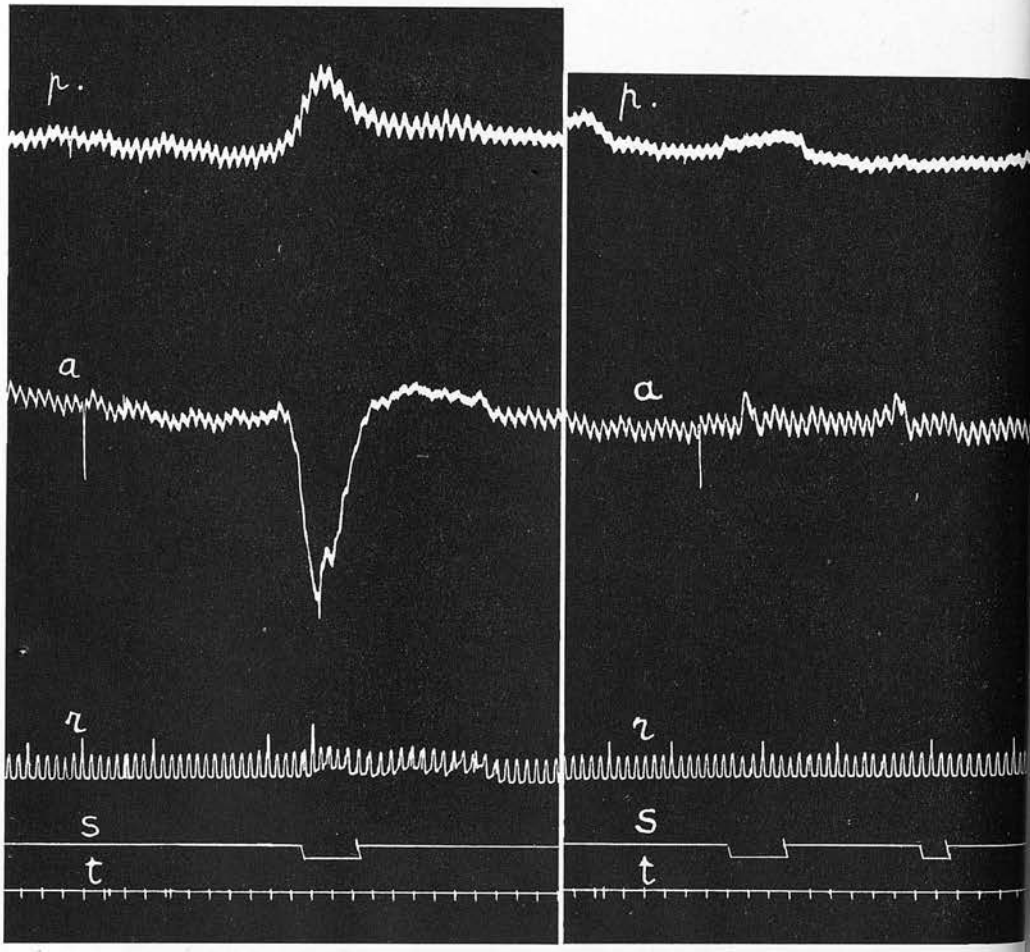


FIG. 4.—Effect of a repetition on the same animal within a few minutes of the same dose of whole-gland extract, *i.e.* extract containing both substances. Immunity is now established for the histamine-free substance, and the effect is entirely that produced by the histamine-like substance.

FIG. 5.—Administration in the same cat minutes later of a dose equivalent to 5 m posterior lobe, which had been extracted alcohol and thus deprived of its histamine. extract is without effect, immunity having established for the histamine-free principle.

extraction with alcohol is given, no effect whatever is seen (fig. 5), since immunity to the histamine-free principle has been produced, and it is necessary to wait for an hour or more before the immunity is recovered

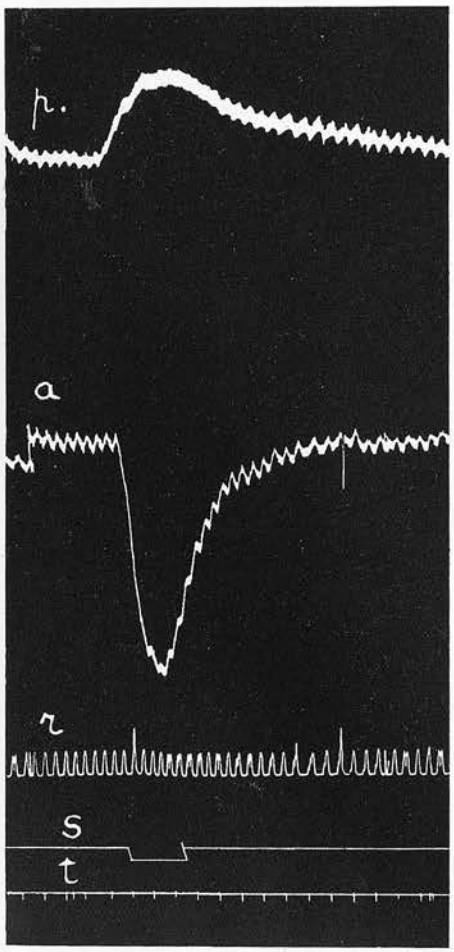


FIG. 6.—Effect on the same animal of an intravenous injection of a dose of Ringer's solution representing 2 mgm. dried alcoholic extract of posterior lobe of pituitary of ox.

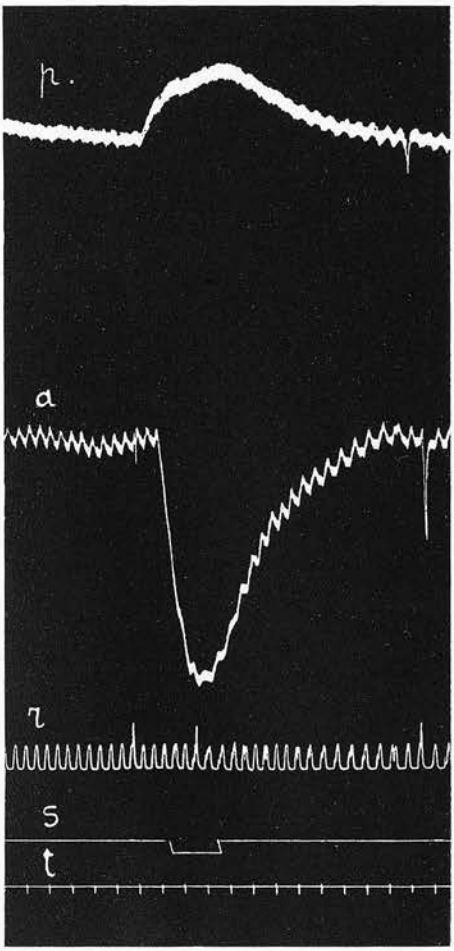


FIG. 7.—Effect of an intravenous injection on the same animal of 0·02 grm. histamine (ergamine acid phosphate). Notice that the effects shown in figs. 6 and 7 are identical, and resemble that of the repeat dose of whole gland shown in fig. 4.

from. On the other hand, the alcohol-extract reproduces the effect of the "repeat" dose of whole-gland substance (fig. 6). And an appropriate dose of histamine exactly reproduces the effect of the alcohol-extract (fig. 7).

If we now turn our attention to the action upon the pulmonary and

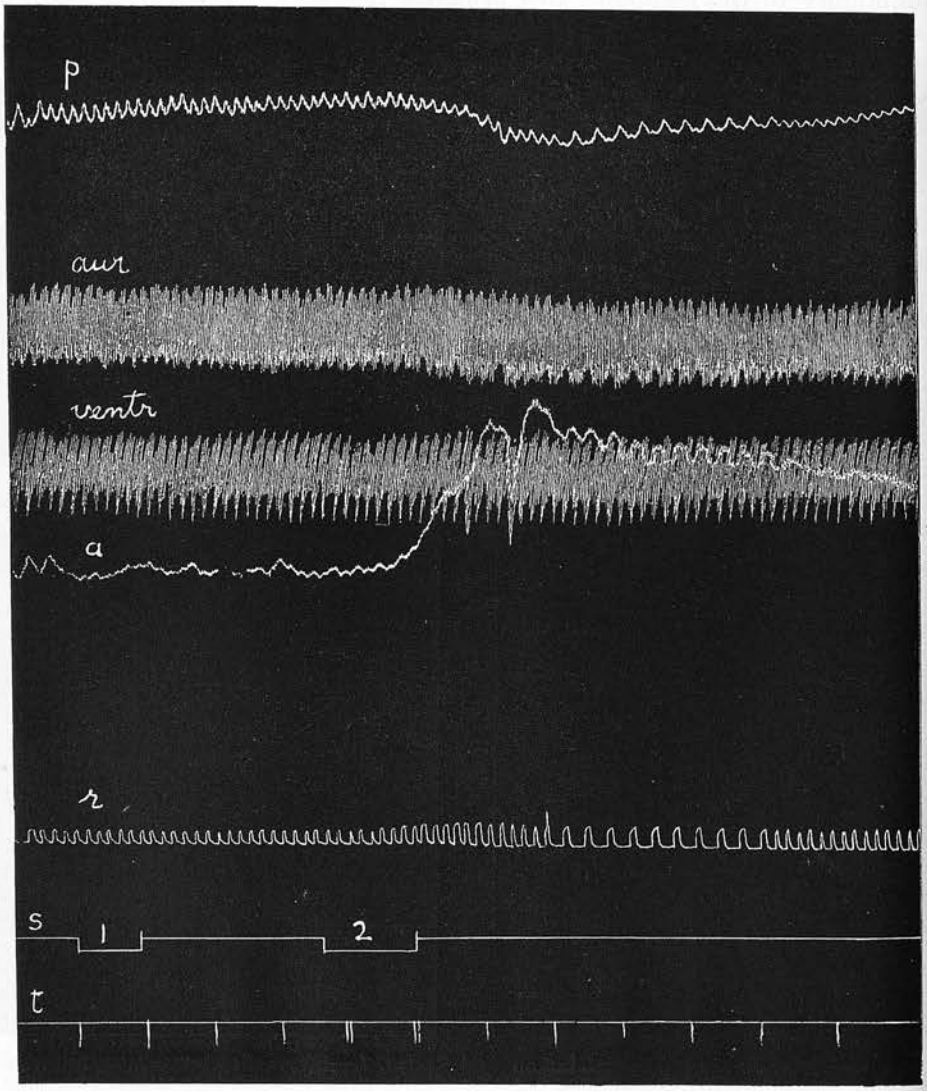


FIG. 8.—Cat, ♂, 2400 grm. Anæsthetic, ether Effects of a first dose of histamine-free substance on pulmonary and aortic pressures, heart and respiration. Lettering as in previous figures.

Notice the rise in aortic pressure, accompanied by a slight fall in pulmonary pressure, with no perceptible effect on the heart. The first signal (1) shows the intravenous administration of 1 c.c. of Ringer given as a control ; the second (2) a similar amount of Ringer extract representing 5 mgm. of alcohol-extracted posterior lobe of ox.

aortic pressures of the histamine-free substance alone, we find certain differences in different animals. In the cat there is usually a prolonged

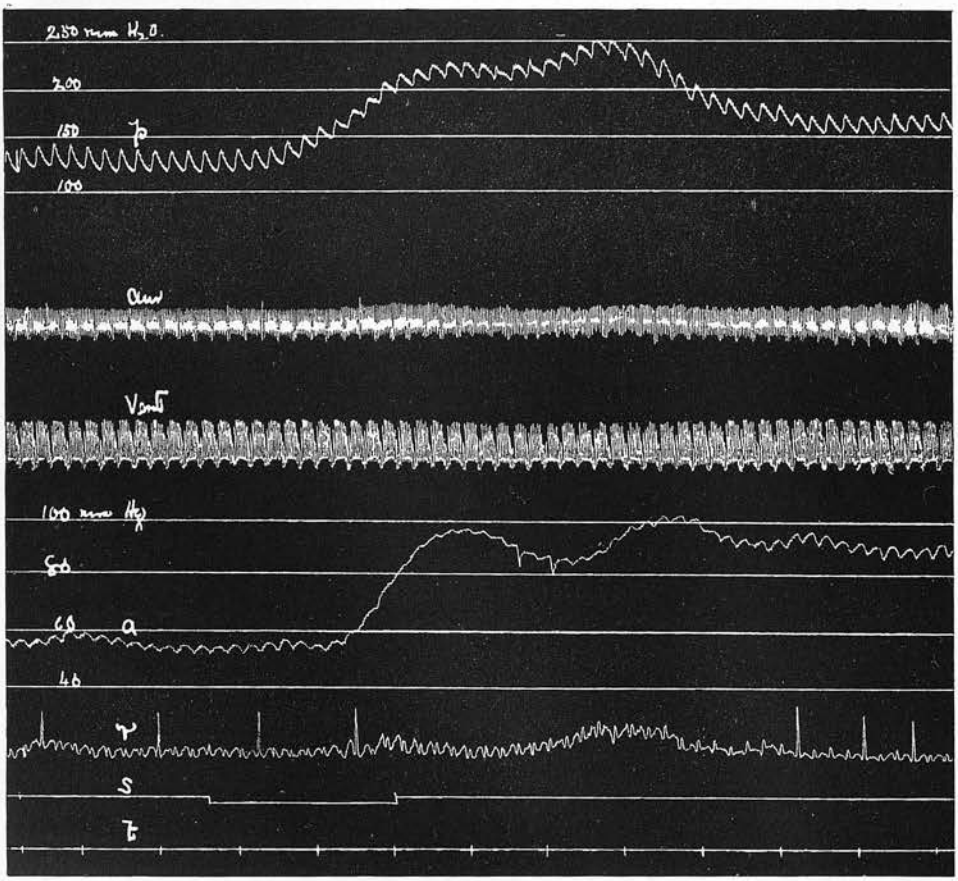


FIG. 9.—Cat, decerebrated. Effects of a (first) dose of histamine-free substance (representing 5 mgm. of dry bovine posterior lobe substance after extraction with absolute alcohol) on pulmonary pressure, aortic pressure, auricle, ventricle, and respiratory movements.

p, pulmonary pressure in mm. H_2O ; *aur*, record from auricle ; *ventr*, record from ventricle ; *a*, aortic pressure in mm. Hg ; *r*, respirations. The artificial respirations are superposed in this on the natural respirations : *s*, *t*, as before. Scales of pressures on left.

Notice the check or "dip" on the rising curves of both pulmonary and aortic pressures. The heart-beats are very slightly slowed and diminished in extent. The respirations appear unaffected except for slight slow variations in muscular tone, but these variations were present, as well as the occasional deep inspirations, before the injection of the extract.

slight fall in the pulmonary system, whilst the aortic pressure is considerably raised (fig. 8). Exceptionally we found in one (decerebrated) cat a well-marked rise in both pulmonary and aortic systems, interrupted by a shallow "dip" (fig. 9). And in another (chloralosed) cat no effect

whatever was produced upon the pulmonary curve whilst the aortic pressure showed a very well-marked rise (fig. 10).[1]

But, as stated, the usual result in the cat of injecting the histamine-free extract is the production of a slight prolonged fall in pulmonary pressure. In the dog (fig. 11) and rabbit (fig. 12) the histamine-free extract also causes a fall in the pulmonary pressure, which may be slight, but is usually distinct, and is occasionally very marked and prolonged, a condition we have not observed in the cat. One reason for the difference seems to be that in the cat the heart is only slightly affected by the histamine-free substance, and sometimes not at all, whereas in the dog and rabbit the heart is invariably slowed and greatly weakened, or, if atropine is given, weakened without slowing (as in fig. 11), so that the beats, especially those of the auricle, are hardly recorded by the cardio-myograph, and it is only on direct inspection that one can assure oneself that the heart is still beating, the right auricle and right ventricle being both much dilated, and the whole heart pulsating very feebly; the weak contractions are evidently insufficient to drive enough blood into the pulmonary system to maintain its usual pressure. In other words, any tendency of the histamine-free substance to cause contraction of the pulmonary vessels is more than antagonised by the weakened condition of the heart; the resultant being a fall in the pulmonary pressure, which is greater or less according as the one or other action obtains the predominance. This cardiac weakening may occur to some extent in the cat also, but not sufficiently to cause so marked a fall in the pulmonary pressure as is seen in the dog and rabbit. Whether such fall in pulmonary pressure as occurs in the cat is due, as it certainly is in the dog and rabbit, to the weakening effect of the pressor substance on the heart is uncertain; for the fall may be seen in the cat even when the cardio-myograph records little or no alteration in the rate or force of the heart-beats (as in fig. 8). This weakened condition of the heart, which is always striking in the dog and rabbit (figs. 11, 14), does not in them confine its effects to the pulmonary circulation. The aortic pressure also falls; usually after a preliminary rise (fig. 12), during which presumably the contraction of the systemic vessels is strong enough to overcome the tendency to fall produced by the feeble contractions of the heart. Administration of atropine, although diminishing this effect upon the heart, does not abolish it; the rate may be brought back to normal, but the weakening is still very apparent. Before long, whether atropine is given or not, the weakening effect upon the heart passes off. The recovery usually shows itself first in the ventricle (fig. 13). As this beats more strongly

[1] A similar condition sometimes occurs with adrenaline, which, as a rule, affects both pulmonary and aortic systems similarly, but occasionally causes a very considerable rise of aortic pressure without any effect on the pulmonary system. *Cf.* Sharpey-Schafer and Lim, Quart. Journ. Exper. Physiol., 1919, xii, p. 192, and figs. 27, 32, and 33.

the pressure rises again in the aortic system, sometimes steadily, sometimes showing slow waves of depression and recovery (figs. 14, 15), somewhat like those which M'DOWALL has described as occurring in perfused surviving blood-vessels. Such waves may also be observed in the pulmonary pressure curve, but less distinctly than in the aortic. Whether they are or are not synchronous in the two systems it is not easy to say ; the rate appears to be about the same. If so, their cause will probably be central rather than peripheral ; naturally the latter must be the case with the waves shown in perfusion curves.

With a sufficient first dose of histamine-free substance, subsequent doses administered within a few minutes are inert (tachyphylaxis) (figs. 5, 16, and 18). To obtain a second effect similar to the first, it is necessary to wait an hour or more for the immunity established by the first dose to pass off (fig. 19). The immunity includes not only the effects produced on the blood-vessels, but also the weakening and slowing effects on the heart (figs. 1, 16). Whether any immunity occurs in perfusion experiments we cannot certainly say, but M'DOWALL mentions experiments (made with whole-gland extracts) which seemed to show it in the rabbit. The phenomenon appears to be correlated with the particular autacoid or hormone which raises blood-pressure, as distinct from the oxytocic and other autacoids which are also present in the residue after extraction with alcohol, for no one of these, so far as is known, exhibits the phenomenon of tachyphylaxis.

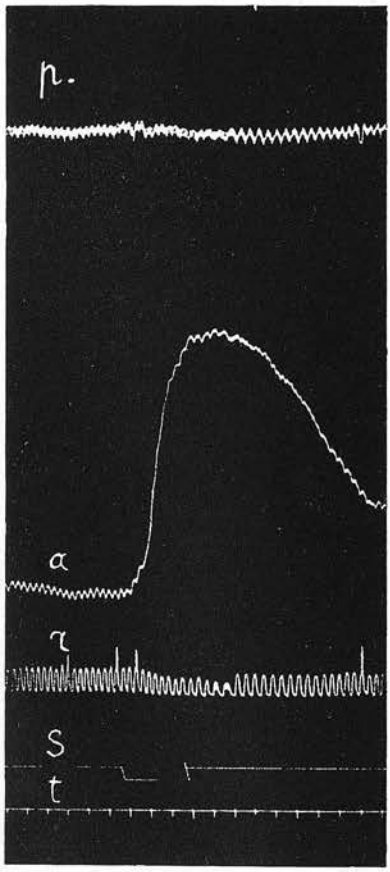


FIG. 10.—Cat, chloralose. Effects of a first dose of 5 mgm. histamine-free substance on pulmonary and aortic pressures and on respiration. In this experiment the thorax was closed after insertion of the pulmonary cannula, and the animal's breathing was carried on by its own respiratory efforts.
There is no effect perceptible in the pulmonary pressure. In the aortic-pressure tracing the rise and fall seem sharper than in some of the tracings, because the rate of the paper was much slower. The respiratory movements are diminished in extent for about forty seconds.

If the first dose is insufficient to produce immunity, a second and even a third dose will cause a repetition of the effect of the first dose, but with a gradual decrement and eventual disappearance, immunity

being now established. As has already been mentioned, such immunity is also obtained if a sufficient dose of whole-gland extract is administered, but the animal is in no way immune to the effects of the alcohol-extract (histamine), for repeated administration of whole-gland extract or of alcohol-extract each time produces the fall in aortic pressure which is

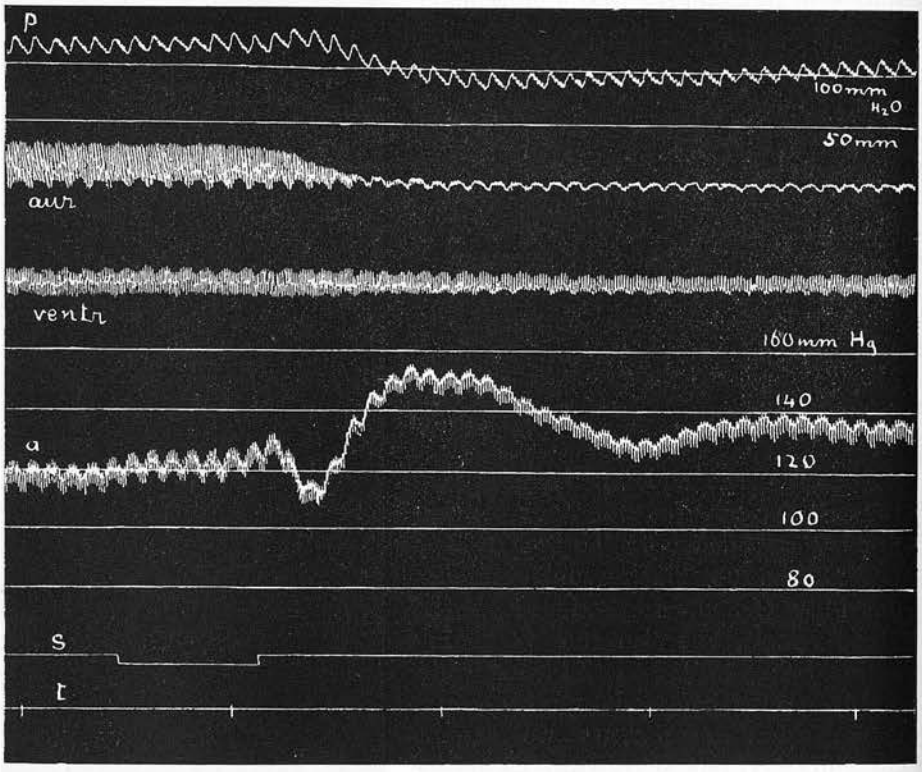


FIG. 11.—Dog, 11 kilos. Chloralose (0·8 grm.); atropine sulphate (0·3 c.c. of a 1 per cent. solution). Effects of a first dose of histamine-free substance on pulmonary and aortic pressures and on heart. The respirations are not recorded. (For continuation of this experiment see figs. 20 and 21.) Lettering as in previous figures.

At the signal, 1 c.c. Ringer-containing extract representing 10 mgm. alcohol-extracted posterior lobe substance was injected into the ankle-vein.

Notice the fall in pulmonary pressure (the slight preliminary rise might be produced by injection of an equal amount of Ringer); the preliminary fall in aortic pressure followed by a rise, and this by subsequent slow fluctuations; the weakening of the heart-beats (without slowing), very marked in the auricle, less marked in the ventricle.

characteristic of a second dose of whole gland, and this is always accompanied by a rise in pulmonary pressure, both being histamine effects.[1] This is well seen in the tracings shown in figs. 20 and 21, which are from the same experiment as that recorded in fig. 11, but taken about twenty minutes later. The heart-beats are not affected detrimentally by the

[1] The rabbit forms an exception to this statement (as to the fall in aortic pressure); in this animal, when anæsthetised, histamine causes a rise.

alcohol-extract, at least in the doses which we have employed ; if anything, the strength is increased.

The histamine effects can also be obtained again and again by repeat doses, each of which causes changes exactly like those caused by the first dose.

There is no doubt that the substance which is extracted by alcohol has the physiological characters of histamine ; although it cannot be pure histamine, for all alcohol-soluble substances would be taken up ;

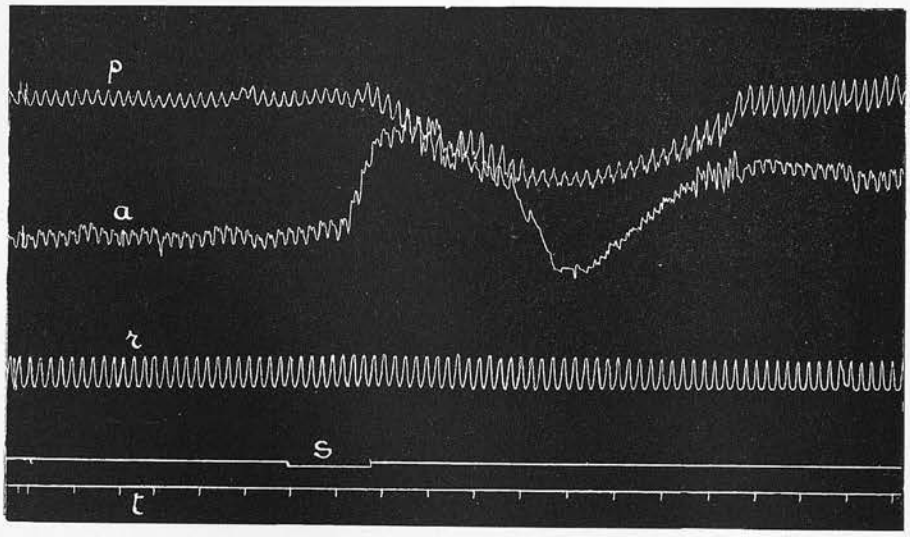


FIG. 12.—Rabbit, ♂, 2200 grm. Chloralose, 0·15 grm. Chest closed ; natural respiration. Effect of a first dose (=5 mgm.) of histamine-free substance. Lettering as before.

Notice the considerable fall (from 155 to 60 mm. H_2O) in pulmonary pressure, and the rise (from 80 to 120 mm. Hg) in aortic pressure, followed by a fall and a subsequent prolonged rise. The respirations show no change whatever.

the extract may include other alcohol-soluble autacoids—assuming that histamine is itself an autacoid.

Whether histamine is naturally present in the gland or whether it is produced immediately after death or removal of the gland from the body, as certain observations by HANKE and KOESSLER (17), and HOGBEN, SCHLAPP, and MACDONALD (8) seem to suggest, is an interesting question. Even if not present during life, the fact that it makes its appearance with great rapidity after death, or after removal of the gland from the body, shows that there must be a precursor present which is easily convertible into histamine. And this conversion may well happen naturally during life, for the conversion cannot be due to putrefactive changes, and is probably a normal enzyme action. It seems, therefore, not improbable that the pituitary body may be an organ for producing histamine, although, since this substance is widely

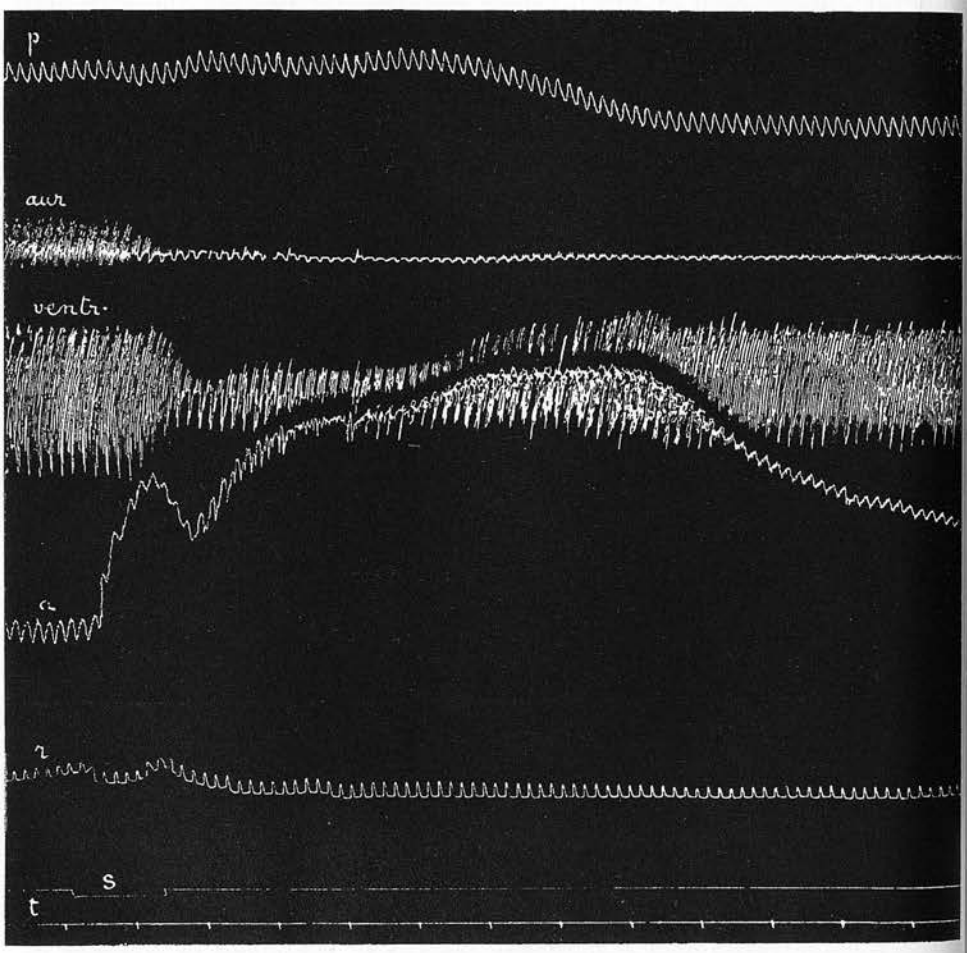


FIG. 13.—Rabbit, ♂, 2300 grm. Decerebrated. Effect of intravenous injection of 1 mgm. histamine-free substance. Lettering as before.

Notice that the fall in the pulmonary pressure (from 120 to 50 mm. H_2O) is deferred for about a minute; the pressure is maintained during that time at a slightly higher level than before the injection. The aortic pressure (originally 60 mm. Hg) rises at first rapidly, is then interrupted by a "dip," and then rises gradually to a great height (170 mm. Hg), then falls gradually. The auricular beats are greatly weakened; this effect lasts for some minutes. The ventricle, on the other hand, is less affected and soon recovers; this can be clearly seen in spite of the accidental interference of the recording pens of ventricle and aortic pressure. The respirations are unaffected, but the slow variations of tone of the respiratory muscles which were exhibited before the injection disappear after it.

diffused in the tissues, it is unlikely to be the sole seat of its production.[1]

The histamine-free extract acts, as we have seen, not only on the blood-vessels but also on the heart, especially in the dog and rabbit,

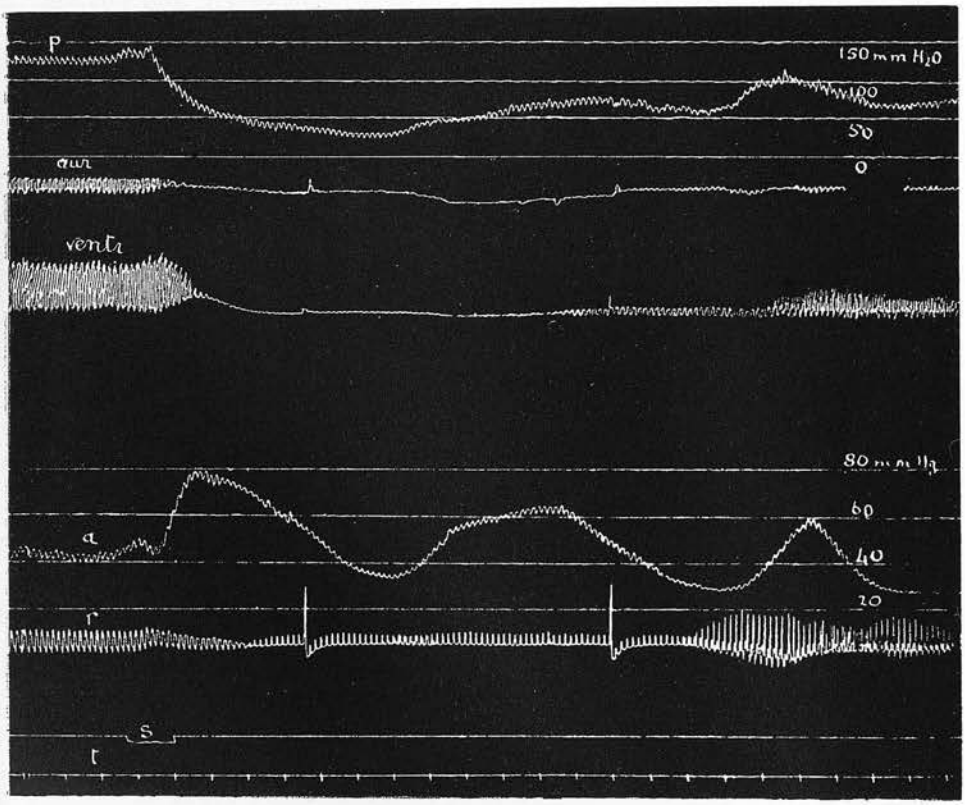


FIG. 14.—Rabbit, ♀, 2250 grm. Decerebrated. Effect of a first dose (=5 mgm.) of histamine-free substance. Lettering as before.

Notice the marked fall of pulmonary pressure followed by a gradual rise with slow fluctuations; the rise of aortic pressure followed by a marked fall, and this by slow wave-like fluctuations; the intense weakening effect on the heart without any apparent slowing; the gradual recovery, showing first in the ventricle. As inspection showed, the heart continued to beat throughout. The respiratory movements were rendered irregular by the extract.

causing marked slowing and weakening, the slowing effect being produced through the vagus. The slowing effect was already remarked by HOWELL (18), and may have the effect of causing a pure fall of aortic as well as of pulmonary pressure (fig. 22). The aortic fall is converted into a rise after administration of atropine (fig. 23).

Although " pituitary " has been long known to cause slowing of the

[1] BURN and DALE (6) record an experiment which seemed to them to indicate the lungs as an important source of histamine, but the conditions of the experiment were such that it might also have been yielded by the pituitary body.

cardiac rhythm, the weakening effect, which is physiologically far more important, seems to have escaped the observation of most previous workers, who have confined themselves to the examination of the results of its injection on blood-pressure. But if the effect of a drug on the heart is to be investigated, it is necessary to expose that organ, and not only to record its contractions but also to observe directly any changes

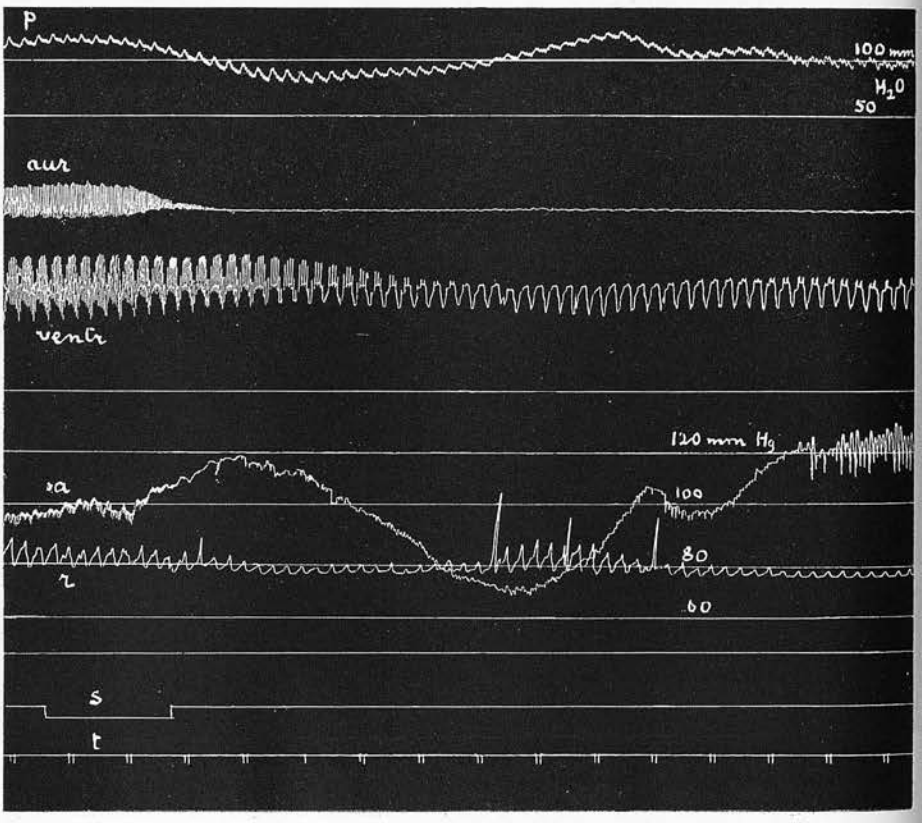


FIG. 15.—Dog, 12 kilos. Morphine—no atropine. Vagi intact. Effect of a first dose (=10 mgm.) of histamine-free substance. Lettering as before.

Notice the fall in pulmonary pressure, succeeded by fluctuations; the rise in aortic pressure, succeeded by a fall considerably below the original level, and this by a rise interrupted by a wave of depression. The heart-beats are greatly weakened and also slowed; they are scarcely perceptible in the auricular tracing. The respiratory movements were irregular even before administration of the extract.

which may be produced. Without this precaution erroneous conclusions may be arrived at. For example, it is commonly stated that pituitary extract not only slows the heart but also increases its force. The increase in force seems to have been deduced from the large cardiac fluctuations seen on the kymographic curve of blood-pressure. But these are not indications of increased force of contraction; they merely indicate the fact that in consequence of a slowing of the rhythm the

blood-pressure has more time to fall between the successive contractions, so that the result of each cardiac contraction appears larger than before, and this suggests that the heart is beating more strongly, although the very opposite may be the case. If records are made with the cardiomyograph, it is seen (figs. 13, 14, 15, 27, 28) that the contractions are not only slower but weaker instead of stronger, and they may be so

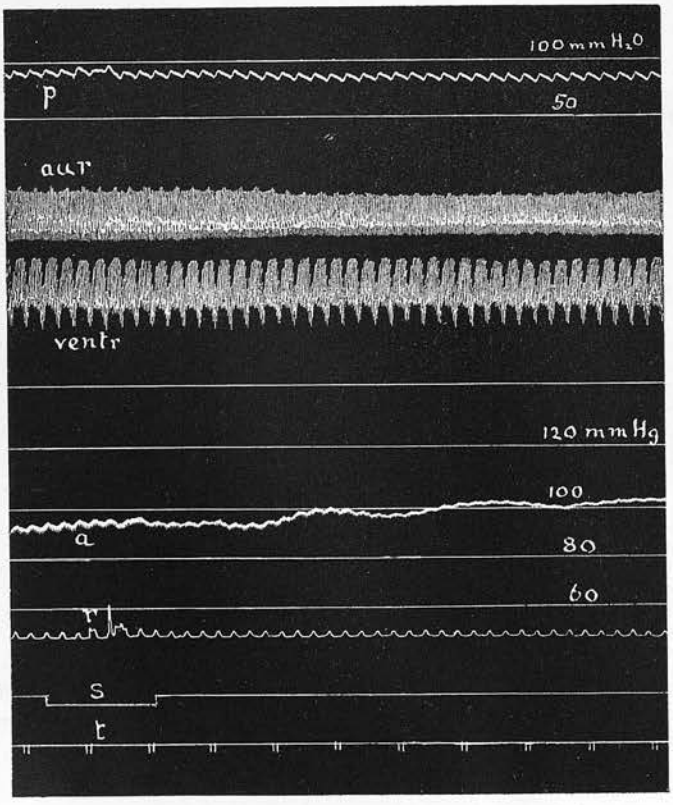
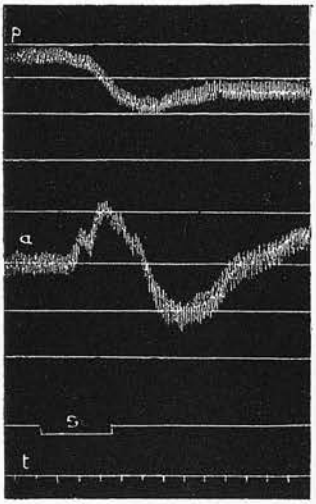
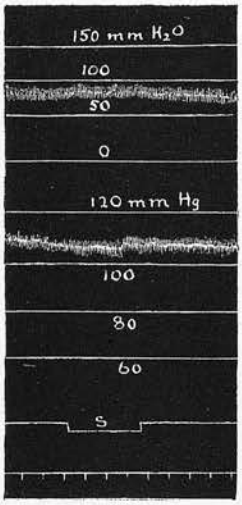


FIG. 16.—From the same dog as that from which the tracings given in fig. 15 were obtained. Effect of a third injection of 10 mgm. histamine-free substance twenty minutes later, showing that immunity (produced by the two previous injections) is established, and affects not only the blood-pressure but also the cardiac contractions. Atropine sulphate (0·2 c.c. of a 1 per cent. solution) had been administered after the first dose, but this, although antagonising the histamine-free substance as regards its effect on the cardiac rhythm, does not abolish the weakening of the cardiac contractions caused by that substance.

greatly diminished in force that, with or without a preliminary rise caused by arterial constriction, the aortic pressure may fall. In the dog this fall is often very striking, as several of the tracings witness, and appears to be the reason why some observers (19) have thought that the effect of pituitary extract is to produce vascular dilatation. As the effect of the extract on the heart passes off, the blood-pressure rises and continues to rise, sometimes with long wave-like fluctuations,

for a considerable time; this being doubtless due to continued contraction of the arteries. When atropine is administered in sufficient amount to abolish vagal action, the above described fall of aortic pressure is not always seen, and the administration of the histamine-free extract may cause only a prolonged rise. In the cat, with the





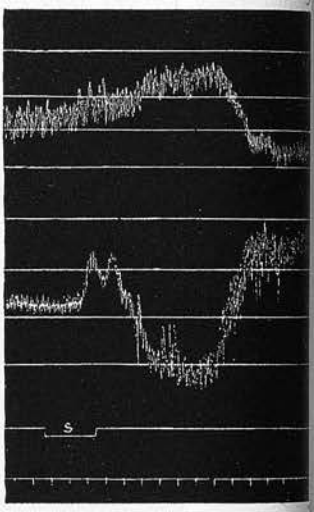


FIG. 17. FIG. 18. FIG. 19.

FIG. 17.—Dog, ♂, 8 kilos. Chloralose, 0·8 grm. Chest closed; breathing natural throughout but with rapid respirations. Lettering as before.

At signal, 1 c.c Ringer solution containing the equivalent of 10 mgm. histamine-free substance injected into ankle-vein.

Notice the fall in pulmonary pressure; the rise in aortic pressure, succeeded by a fall (with slowed cardiac rhythm), and this by a second gradual rise.

FIG. 18.—From the same animal eight minutes later. Effect of a similar dose. Immunity is now established, and the same dose of histamine-free substance produces no effect.

FIG. 19.—From the same animal 1½ hours later. A similar dose of the same extract given. The immunity has passed off and the effect of the first dose is repeated, except that the fall in pulmonary pressure is deferred, being preceded by a rise corresponding with the fall in the aortic pressure.

doses employed, the cardiac weakening and slowing is either absent or comparatively little marked, and the rise in systemic pressure is uninterrupted by a fall even without atropine.

RESNIK and GEILING (21) have made a special study of the effects of post-pituitary extract on the heart, but, in common with most previous observers, have not differentiated the effects of the true pituitary autacoid from those of histamine. They deal mainly with the slowing produced by the extract on the hearts of trained unanæsthetised dogs, the extract being injected into a vein of the fore-limb and the heart's action recorded by an electrocardiograph. Both with and without atropine they find that there is at first produced an increase of rate of the heart, then a con-

siderable decrease in rate, followed after 15 to 30 minutes by a secondary increase. They suggest that the results may be due to the effects of the extract on the coronary arteries or perhaps to anoxæmia affecting the myocardium; but they do not appear to have noticed the extreme weakening of the heart's action which is

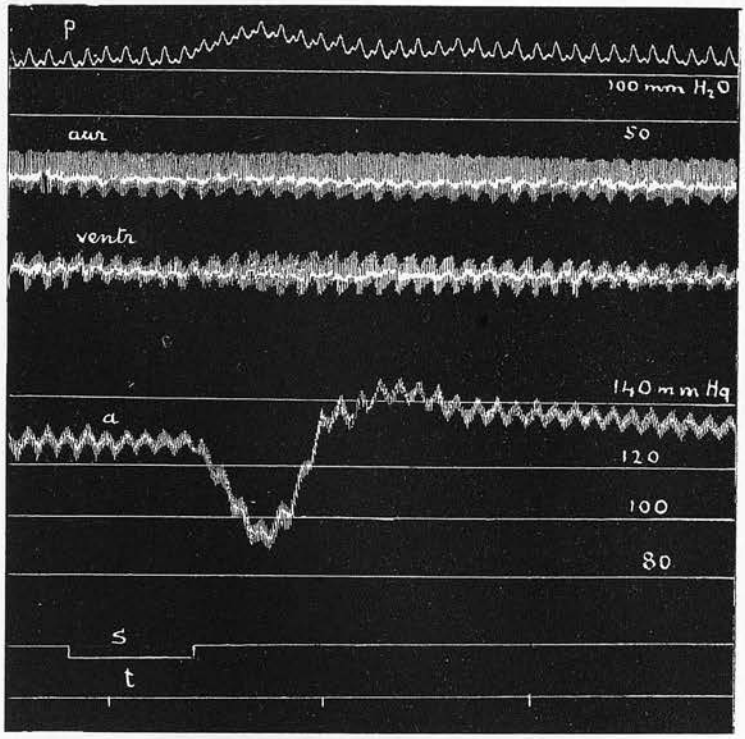


FIG. 20.—Dog, ♂, 11 kilos. Chloralose; atropine. This is a continuation of the experiment recorded in fig. 11, and shows the effect of the injection of 5 mgm. alcohol-extract of dry posterior lobe in 1 c.c. Ringer

Notice the rise in pulmonary pressure and the fall, with subsequent rise, in aortic pressure. The action of the heart is not weakened as with the histamine-free substance, but is even somewhat strengthened.

recorded above. They obtained with the pituitary tartrate of Abel and Geiling results similar to those yielded by the crude extract (22).

In the species investigated by us, *e.g.* cat (fig. 24), dog (figs. 17 and 25), and rabbit (fig. 13), it sometimes happens that the first rise is followed by a depression ("dip"), succeeded by a further prolonged rise. The depression is not very unlike the curve of blood-pressure which is caused by splanchnic stimulation (20), which shows a rise interrupted by a "dip" with a prolonged further rise; this result is usually ascribed to outpouring of adrenaline from the supra-

renals. In order to test whether the effect shown in fig. 24 was due to secretion of adrenaline or not, we subsequently tied off both suprarenals and, having waited an hour and ten minutes, which we considered to be sufficient time for the immunity caused by the first dose to pass off, we administered another dose of the histamine-free extract. This now produced an uninterrupted rise in both aortic and pulmonary pressures without any depression in the aortic curve

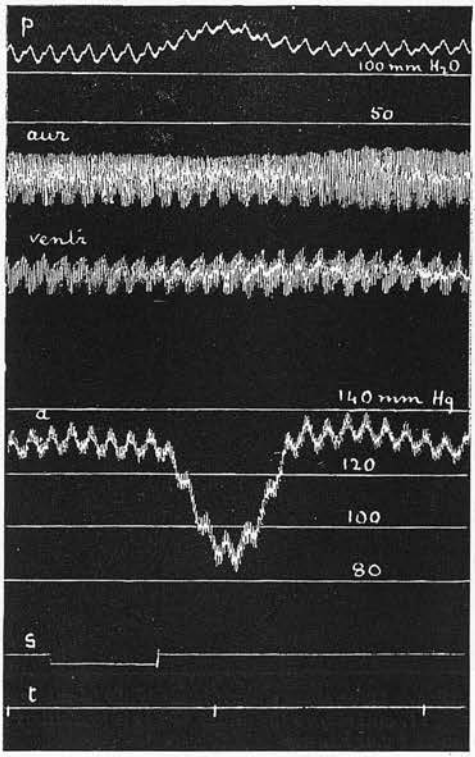


FIG. 21.—This is also a continuation of the same experiment, and shows the effect of injecting 0·05 mgm. histamine (ergamine acid phosphate), which almost exactly reproduces the effect of the alcohol-extract shown in fig. 20.

(fig. 26). In another experiment, in which we tied off the suprarenals before giving a dose of pituitary at all, the result of a first dose was exactly similar to the curves shown in fig. 26. This looks as if the interruption in the rise of the aortic curve were due to adrenaline secretion excited by the histamine-free substance, but more evidence is necessary before a positive opinion can be given. The difficulty in all experiments with this substance is that the immunity established by a first sufficient dose interferes for so long a time with the effect of subsequent doses. This necessitates very prolonged experiments, and the animal is not easily maintained in a satisfactory condition

throughout. It may be remarked that in the two experiments upon the cat on the action of histamine-free extract after the suprarenals had been tied off, not only was the " dip " in the rising aortic pressure abolished but the usual fall in pulmonary pressure was converted into a rise. The explanation of this change is difficult, unless we are to suppose that it indicates that the slight fall in pulmonary pressure which is usually seen in the cat is caused by the action of a small

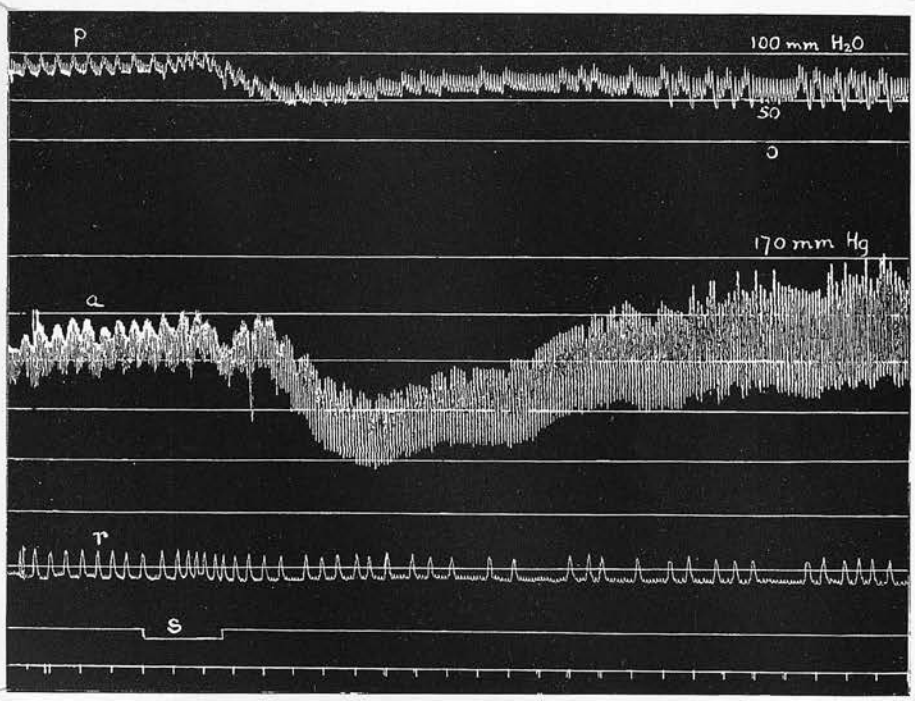


FIG. 22.—Dog, ♀, 6 kilos. Chloralose (0·5 grm.) given by the mouth in milk. The effect of 5 mgm. histamine-free substance upon pulmonary and aortic systems is seen in this case to be purely depressor on both, largely owing to its slowing action on the heart.

amount of adrenaline shed out under the influence of this extract. A rise in pulmonary pressure is also noticeable in the experiment on a dog (fig. 19) in which a " repeat " dose of histamine-free substance was given after the immunity caused by earlier doses had passed off. In this animal the suprarenals were intact.

We have made one experiment in a rabbit, in which, instead of tying off the suprarenals, we paralysed the sympathetic system by a dose of ergotamine tartrate and then injected a small dose of histamine-free extract. The result of this experiment is shown in fig. 27, from which it is seen that the pulmonary pressure shows no rise but a pronounced fall ; the aortic pressure a sharp rise without any " dip "-

like interruption, followed by a pronounced fall, and this again by a gradual and prolonged rise ; the heart-beats, both auricular and ventricular, are slowed and weakened. But the absence of the " dip " on the rising aortic curve may occur without ergotamine, as is seen by a comparison with the tracing on fig. 28, obtained from another rabbit, with a similar dose of histamine-free extract and without ergotamine. This shows very well the prolonged fall in pulmonary pressure, the sharp rise in aortic pressure, followed by a gradual fall, and this again

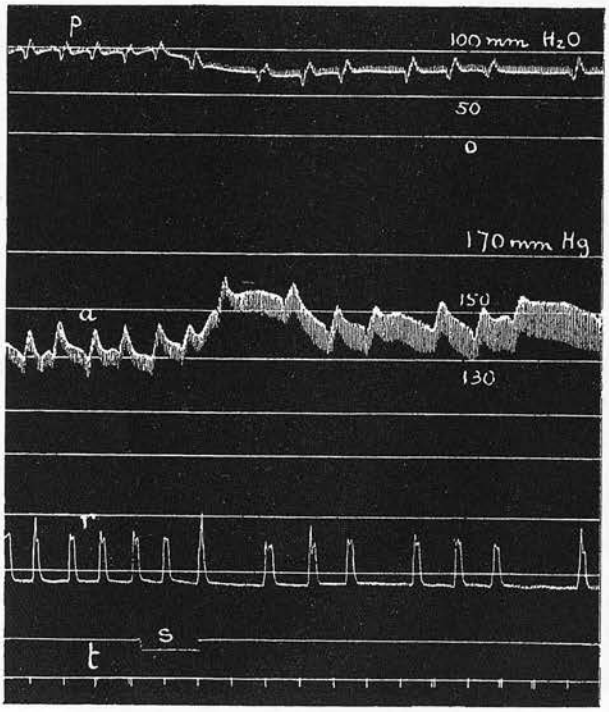


FIG. 23.—Effect in the same animal of a subsequent dose of 5 mgm. of the same extract, the vagi having been cut and atropine sulphate (0·2 c.c. of a 1 per cent. solution) administered prior to this tracing being recorded. There is still a fall in pulmonary blood-pressure, but a rise in aortic pressure is substituted for the fall shown in fig. 22.

by a very marked and prolonged rise. It seems evident from these curves that the weakened condition of the heart is responsible for the considerable fall which occurs in the dog and rabbit in both pulmonary and aortic pressures, but that as regards the aortic pressure the contraction of the systemic arteries is sufficient at first to overcome the weakening of the heart.

The cause of the weakened action of the heart is obscure. That it is not due to the direct effect of the histamine-free extract on the cardiac musculature is evident from the fact that comparatively little weakening is seen when the same extract is tested on a rabbit's heart perfused by Langendorff's method.

The weakened condition is associated with dilatation of the right cavities, and this is probably due to obstruction of the blood-flow through the lungs. *Prima facie* such obstruction should produce a rise in pulmonary blood-pressure, rather than the fall which usually occurs. But it must be remembered that the result of such an obstruction would be to diminish the amount of blood flowing to the aortic system and to the heart-muscle itself by way of the coronaries. This appears the most probable explanation

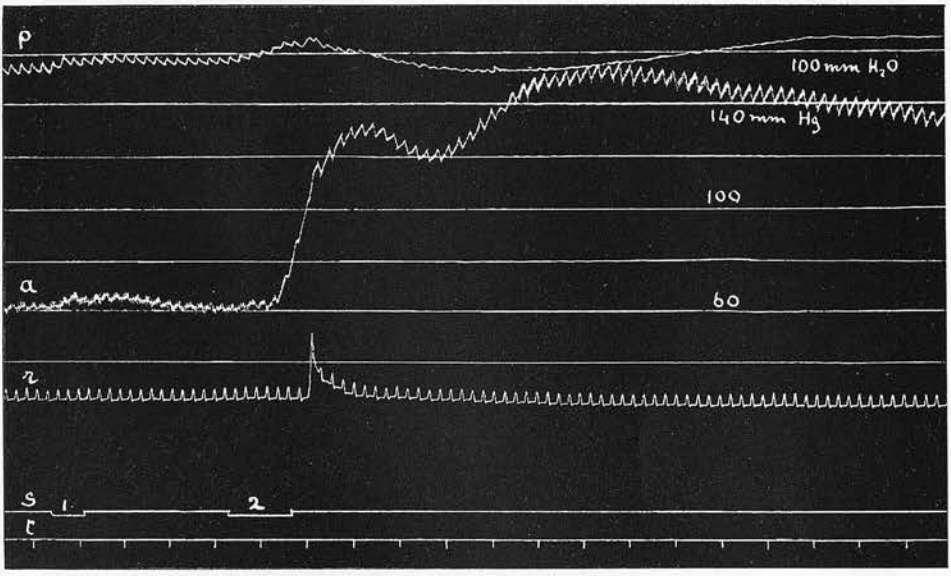


FIG. 24.—Spinal cat, ♀, 3100 grm. Effect of a first dose of extract of 5 mgm. histamine-free posterior lobe. The first signal indicates the injection of 1 c.c. Ringer as control, the second the same amount of Ringer containing the extract.

Notice the slight rise succeeded by a slight but prolonged depression in the pulmonary curve ; the sharp rise in the aortic curve followed by a "dip," and this by a prolonged rise followed by a very gradual fall. The respiration curve records artificial respirations ; it is interrupted immediately after the injection by a convulsive movement.

of the cardiac weakening. That such weakening affects the whole heart, the left ventricle as well as the right, is apparent from the striking secondary fall of pressure in the aortic system (figs. 11, 14, 15, 27, 28).

The pulmonary obstruction is not an asphyxial effect, since the dilatation of the right cavities occurs with artificial respiration. It is possible that it is caused by contraction of pulmonary veins ; in that case the pulmonary capillaries would fill with blood, and there might be little or no initial rise in the pressure in the pulmonary artery.

Summary.

1. The effect on the circulation of the substance separable by alcohol from the posterior lobe of the bovine pituitary, and the effect of the residue after such separation, has been examined in many experiments on the cat, dog, and rabbit, and in one experiment on the monkey.

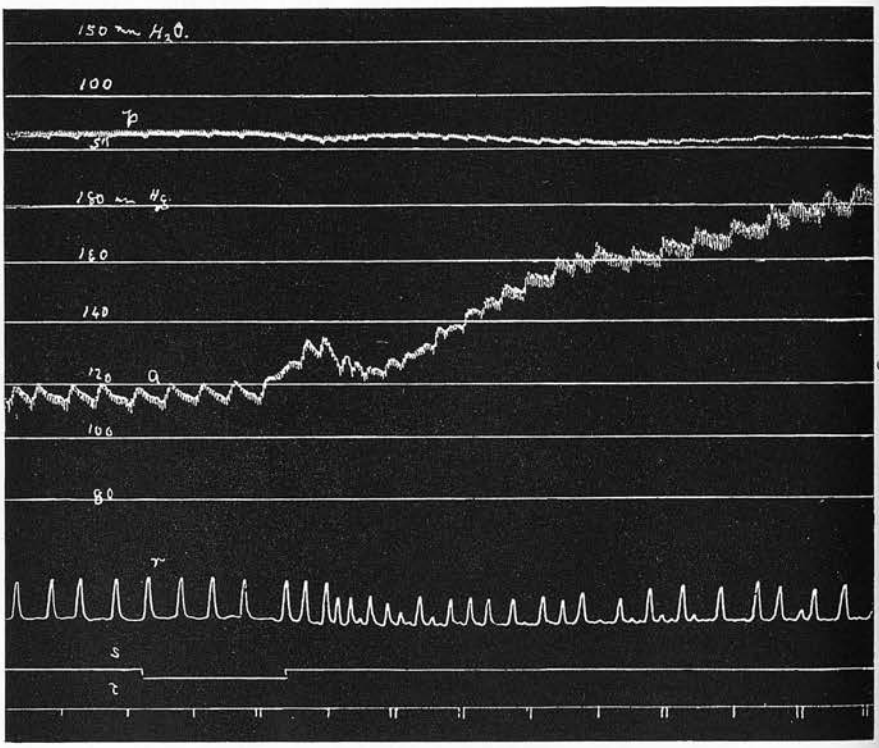


Fig. 25.—Dog, ♂, 8 kilos. Morphine sulphate; atropine sulphate. Effect of injecting (into ankle-vein) 1 c.c. Ringer containing extract of 5 mgm. histamine-free post-pituitary substance. This was the second dose in this animal; the first dose produced a similar but more pronounced effect.

Notice the slight fall in pulmonary pressure and the rise in aortic pressure interrupted by a "dip." The respirations, which were very regular before the injection, are rendered more rapid and somewhat irregular.

2. The alcohol-extract, when dried and dissolved in water, reproduces in all respects the action of histamine.

3. The residue after extraction with alcohol, when dried and dissolved in water, differs somewhat in its action in the species examined.

4. In the cat, extracts of histamine-free residue always cause a rise of aortic pressure, and generally a slight fall of pulmonary pressure as well. Occasionally there is no effect produced on the pulmonary pressure; and in one experiment on a decerebrated cat the fall was replaced by a rise, relatively as great as that produced in the aortic pressure. In all

animals the rise in aortic pressure is often interrupted by a " dip " somewhat like that caused by stimulation of the splanchnic. This " dip " we have found to be abolished in the cat after tying off the suprarenal capsules ; a dose of pressor substance then causes an uninterrupted and prolonged rise in both pulmonary and aortic pressures. We have, however, performed an insufficient number of experiments to prove conclusively that the " dip " is due to the secretion of adrenaline.

5. In the dog and rabbit the histamine-free substance always produces a distinct fall in pulmonary pressure, often very well marked.

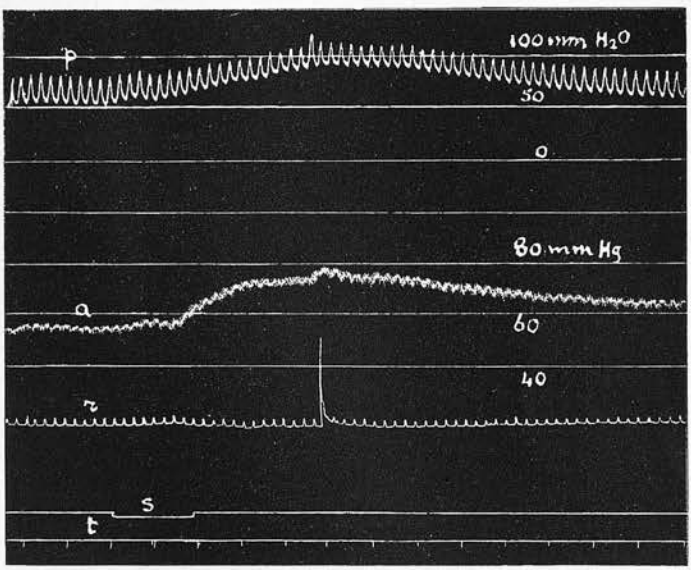


FIG. 26.—This tracing is from the same spinal cat as that used in the experiment illustrated in fig. 24, but in the interval both suprarenals had been tied off. It shows the effect of administration of another dose of 1 c.c Ringer containing the extract of 5 mgm. histamine-free post-pituitary substance. There is now produced a gradual rise in both curves without any " dip " on the aortic curve. The irregularity in the middle of each curve is caused by a convulsive movement. This was not the effect of the extract, for similar convulsive movements were occurring occasionally before the administration.

In these animals the aortic pressure first rises rapidly and then falls even below the original pressure. The fall in question is not to be confused with the above-mentioned " dip " in the curve of rising aortic pressure, which may or may not occur as well. After the fall the aortic pressure rises steadily and progressively, and may attain a great height, the pulmonary remaining low. In some cases this progressive rise is replaced by a series of slow and large fluctuations in pressure, somewhat like those which have been described in perfused surviving vessels.

6. Some of these differences are explained when the effects produced on the heart are observed and recorded. In the cat, the effect on the heart is generally slight and may be absent ; in the dog and

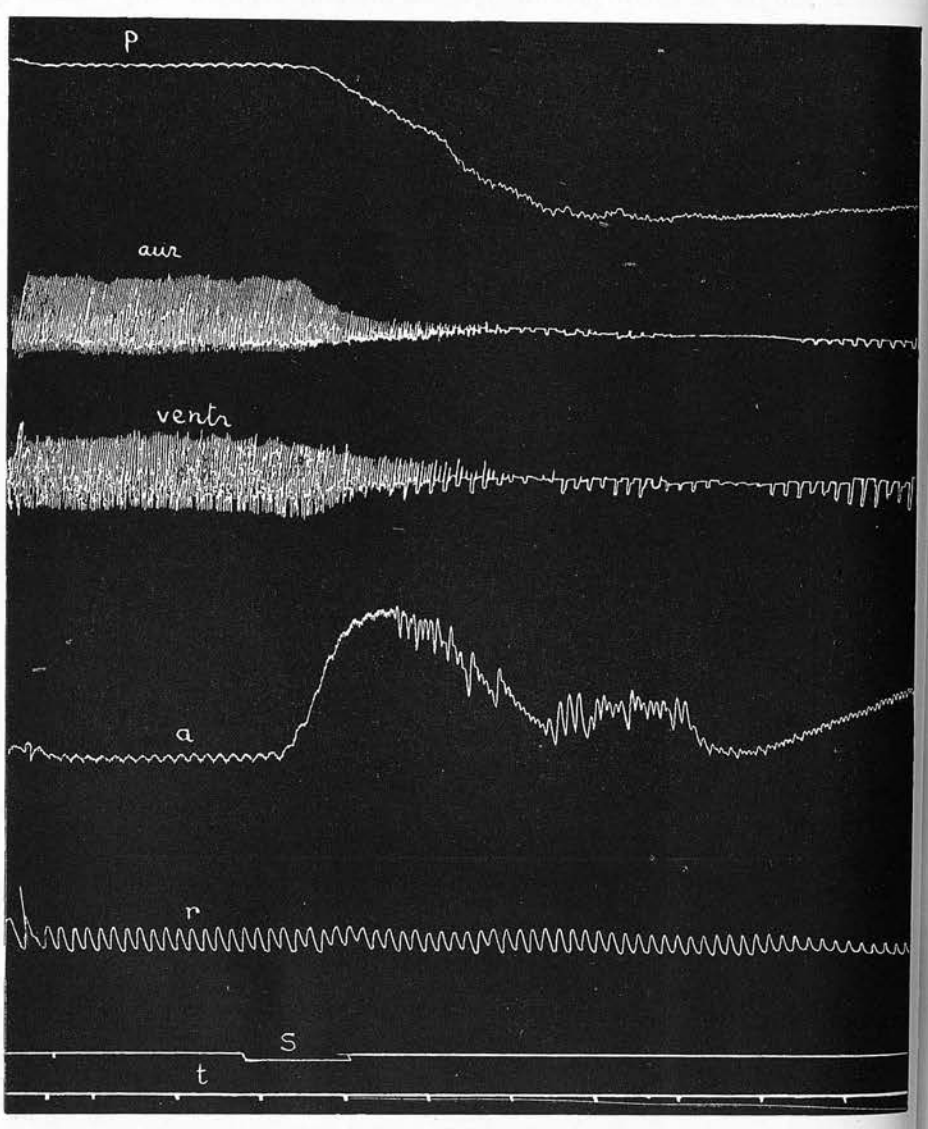


FIG. 27.—Rabbit, ♂, 2000 grm. Sympathetic paralysed by ergotamine tartrate. The completeness of the paralysis was determined by absence of response to adrenaline. (This tracing is to be compared with the next.)

Effect of intravenous injection of 1 c.c Ringer extract representing 1 mgm. of histamine-free post-pituitary substance.

Notice the considerable fall of pulmonary pressure, which is even more pronounced than usual in the rabbit; the rise in aortic pressure followed by a gradual fall (with irregularities), and this by a prolonged rise, only the beginning of which is included in the figure. That the fall in both pressures is accounted for by the slowing and weakening of the heart seems evident from the tracings.

This animal was decerebrated. Similar results are obtained with the "spinal" rabbit.

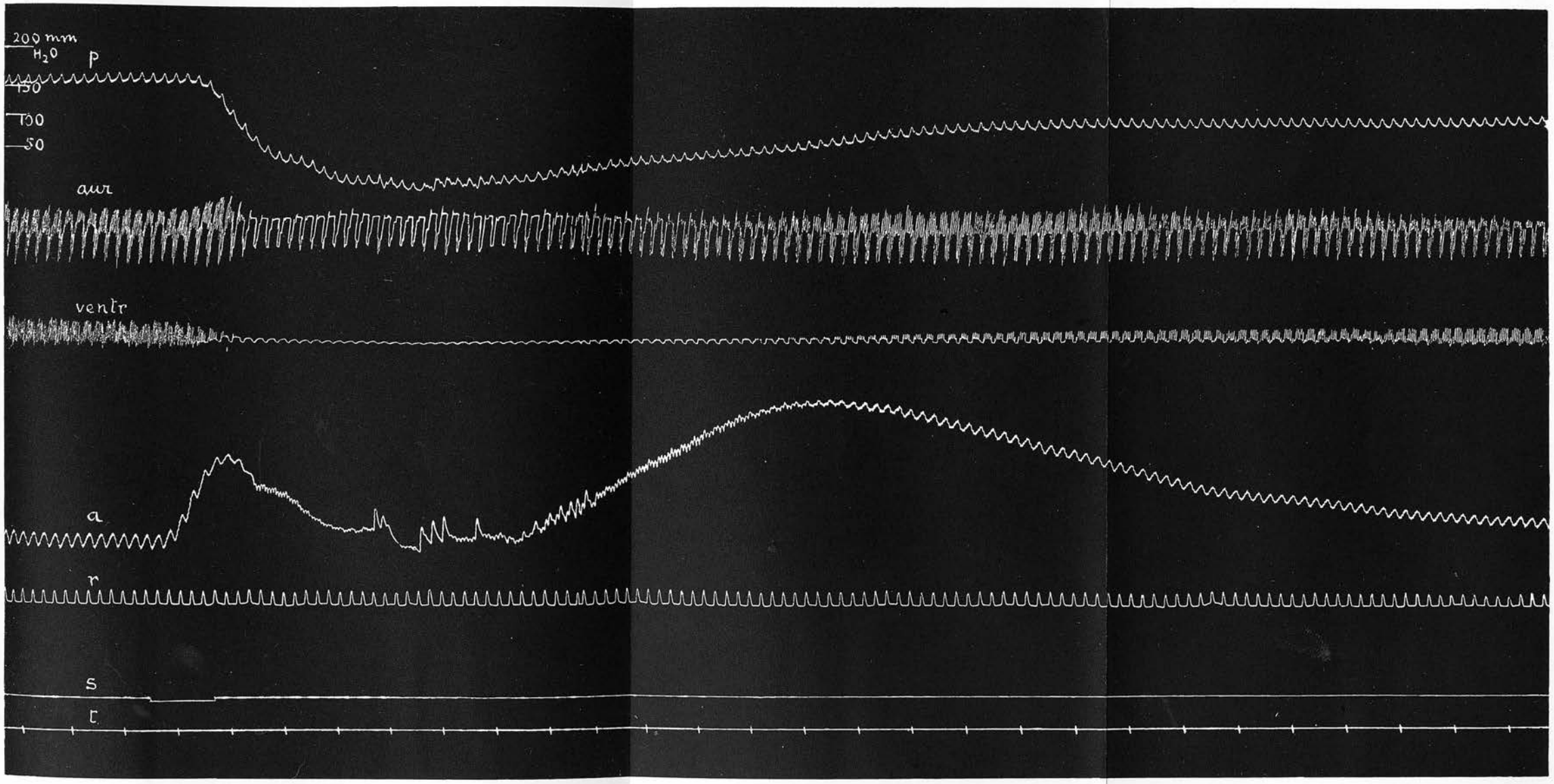


FIG. 28.—Rabbit, ♂, 1950 grm. Chloralose, 0·2 grm. Effect of injection into the jugular vein of 1 c.c. Ringer containing the extract of 1 mgm. histamine-free post-pituitary substance. This experiment was performed under the same conditions as that shown in fig. 27, except that the animal was not decerebrated, nor was ergotamine administered. It will be seen that the effect of the histamine-free substance is practically identical in the two cases. The tracings in both figures are reduced in the same proportion.

(The record of the auricular contractions has been somewhat interfered with by the inflation of the lungs by the respiratory pump, but the recovery of the auricle—which in this case occurs at least as soon as that of the ventricle—can be made out in spite of this interference.)

rabbit the heart is slowed and markedly weakened, and although the slowing may be abolished by atropine the weakening is not thereby abolished, or at least far less readily.

7. It is well known that the effect of a first adequate dose of histamine-free substance in producing contraction of the blood-vessels is not repeated as the result of subsequent doses given within a certain time after the first dose (tachyphylaxis). This, we find, is also true for the effects on the heart. In other words, the result of a first sufficient dose of this substance, both upon the blood-vessels and heart, is to establish immunity to the effect of subsequent doses, an immunity which requires a considerable time to pass off.

8. In the single experiment which we were able to make upon a monkey (the animal had already been used for demonstrating the effect of excitation of the cerebral hemispheres, and was decerebrated for the pituitary experiment), we obtained results which resemble those shown in the cat.

As the tracings which accompany this paper show, we have not obtained any constant result on the respiratory movements with the doses employed, either from injection of decoction of the alcohol-extracted substance or of the alcohol-extract. Such results as are observed in the tracings may perhaps be explained as the effect of alterations in blood-flow in the medulla oblongata.

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The action of pituitary extracts upon isolated blood-vessels.

By E. D. PORTMAN and A. D. MACDONALD. (*Manchester.*)

The diuresis provoked by intravenous injections of extracts of the posterior lobe of the pituitary body in the anæsthetised or decerebrate cat is associated with kidney dilatation. In the study of this volume change much has been made of the dilator action of the extracts on the renal artery(1, 2) while on all other arteries tested varying degrees of constriction are obtained. While such relaxation would not contribute significantly to the plethysmographic changes directly, it might increase the blood supply to the kidney. In the perfused kidney, it must be remembered, pituitary extracts proved constrictor and antidiuretic (Dale(3)).

We have used chains of six rings cut from arteries and veins of the cat, rabbit, dog and sheep, and have found that purified posterior lobe extracts (*i.e.* free from the depressor or histamine-like substance), though very rich in both pressor and oxytocic principles and though producing marked kidney dilatation and a copious diuresis in the cat, are without action alike upon the carotid or femoral and renal arteries and veins, even using concentrations as high as 0·05 p.c. of dried gland. The renal vessels were investigated throughout their lengths, including their first branches. That the absence of response to such extracts is not due to over-weighting or a lack of sensitivity of the preparations is proved by their free contraction to such a stimulus as adrenaline or histamine in a concentration of one in five to ten millions. We are at a loss to explain the dilatation of the renal artery described by earlier observers, unless it be due to the use of commercial or acid extracts.

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THE ACTION OF PITUITARY EXTRACTS ON THE KIDNEY.

By A. D. Macdonald. From the Department of Pharmacology, University of Manchester. (With seven figures and two tables in the text.)

Introduction.

The diuretic action of extracts of the posterior lobe of the pituitary body, when administered intravenously to the anæsthetised animal, as first described by Magnus and Schafer (12), is a readily demonstrable and impressive phenomenon. The anti-diuretic effects in the unanæsthetised animal (Römer (16)) and in man (Farini (4) and Von der Velden (21)), though less dramatic, are equally well established. The apparent paradox involved in the therapeutic development of the use of a drug for a purpose diametrically opposite to the action it was previously shown to elicit, has not failed to exercise both physiologists and clinicians.

Most of the contributions to the literature of the subject give a brief résumé of the findings and conclusions of earlier workers, without attempting explanations or reconciliations of discrepancies in the results (2, 20). The literature up to 1925 is reviewed by Sharpey-Schafer (17). Its consideration presents certain problems, and it is proposed in this paper to summarise the evidence obtained in a long series of experiments directed to the clearing-up of some of the difficulties. The chief controversy has been as to whether the diuretic action is secondary to general circulatory changes, or whether it is due to a true stimulation of the secretory function of the kidney. There is also some argument as to whether both diuresis and anti-diuresis can be produced in all the common experimental mammals, and as to the part played by the anæsthetic and the significance of the method of administration of the extract in the determination of the response.

The Active Principles concerned in the Effects on the Kidneys.

In spite of the clear-cut results obtained by Schafer and Vincent in 1899 (19), many later observations were made with unpurified or commercial extracts, which contained such a non-specific yet powerful pharmacodynamic agent as histamine. The presence of such substances inevitably complicates every reaction exhibited by the gland-extracts, and attention was redirected to the desirability of their removal in the papers by Hogben and Schlapp (6), and Hogben, Schlapp, and Macdonald (7). Again, it was demonstrated by Macdonald (9) that some of the commercial extracts are so acid that even when freely diluted they affect by change of pH alone a tissue suspended in Ringer solution or a perfused heart, and may thus influence the results. For example: 1 c.c. of a commercial extract may increase the acidity of 100 c.c. of Clark's Ringer from pH 8·4 to pH 6·4.

It may be stated categorically that both diuresis and anti-diuresis can be produced with histamine-free laboratory extracts. While anti-diuretic effects have been obtained with both oxytocic and pressor fractions, as separated by Kamm, diuresis is only seen, in my experiments, with preparations and doses which affect the circulation.

The oxytocic fraction has only about 5 per cent. of the anti-diuretic activity of the pressor fraction, and this it may owe to the fact that the separation is not quite complete (5). Recently it has been shown by Kamm and his fellow-workers (8) that it is possible to derive from the pressor principle a powerful anti-diuretic agent which is free from pressor activity, but it is not maintained that this necessarily exists in the gland as a separate substance.

Effects on Isolated Blood-vessels.

The dilatation of the kidney seen in the plethysmographic records of Magnus and Schafer, resulting from the administration of pituitary extracts, suggested the possibility that the renal vessels differ from the others in their response, and might relax. Such relaxation in isolated renal vessels has been detected by several workers, but the published tracings were not very convincing, and the point was considered worthy of reinvestigation (preliminary note, 15). In a series of 106 experiments on isolated arteries and veins, 77 of which were renal, the 29 controls being femoral or carotid vessels, the reactions were studied using depressor-free, neutral extracts. To summarise the results, some relaxation was perceptible in 12 experiments (10 renals), constriction in 16 (14 renals), while 76 (53 renals) gave no effect. Before an experiment was accepted as satisfactory it was shown that the preparation could contract, *e.g.* to adrenaline or to histamine or to both,

and could relax, on reducing the pH of the Ringer in the bath. In the 2 renal experiments not accounted for the arteries proved unresponsive to any drug, and it is assumed that they must have been stretched or otherwise damaged in preparation. The vessels used were obtained from laboratory animals (cat, 20; rabbit, 12) and from fresh slaughter-house material (sheep, 58; cow, 13; pig, 3). A chain of arterial rings

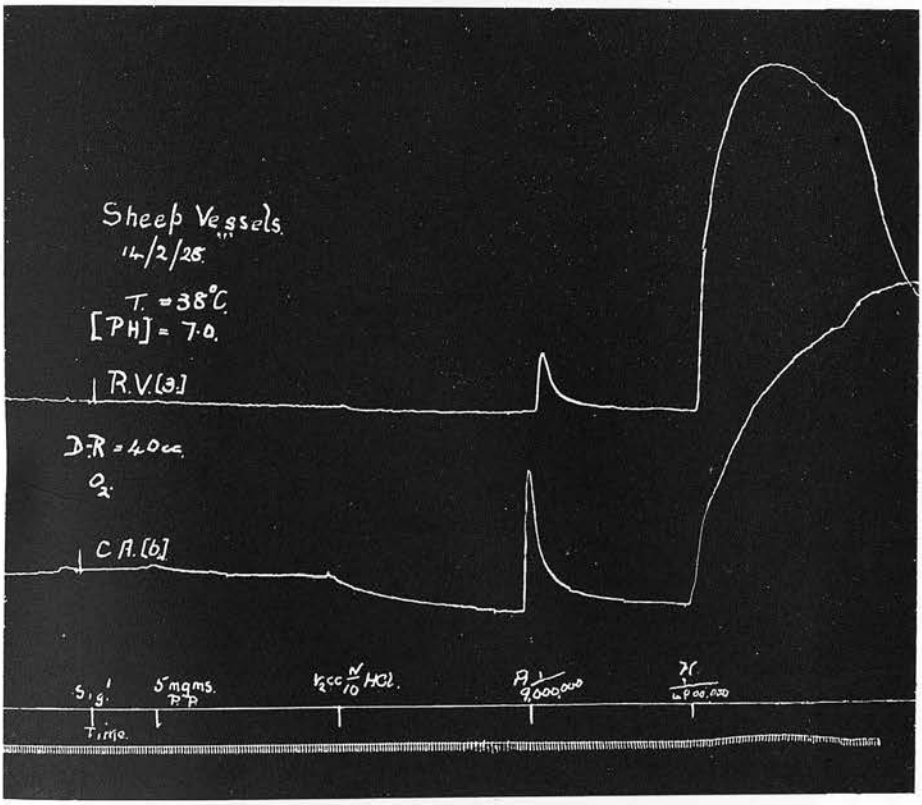


FIG. 1.—*Sheep-vessels.*—Record of the actions of extract of 5 mg. standard posterior lobe pituitary powder, 0·5 c.c. N/10 HCl, adrenaline hydrochloride to give a concentration of 1 in 9,000,000, and ergamine acid phosphate 1 in 4,000,000.

Upper record, 3 rings of renal vein: lower record, 6 rings of carotid artery—each bath containing 40 c.c. Time in 10 seconds.

was prepared as recommended by PAL (14) and suspended in oxygenated Ringer-Locke. Usually when the rings are placed under a tension of one to four grams (according to size) there is a slow relaxation for about 2 hours. In the case of the renal arteries from the larger animals the proximal branches were often used, but most of the experiments were carried out on the main renal vessels.

Typical experiments on sheep-vessels are shown in figs. 1 and 2. Fig. 1 shows, with pituitary extract, a slight relaxation in a carotid artery,

and no effect on the renal vein. Both vessels are relaxed by acid and contracted by adrenaline and histamine. Fig. 2 compares the responses

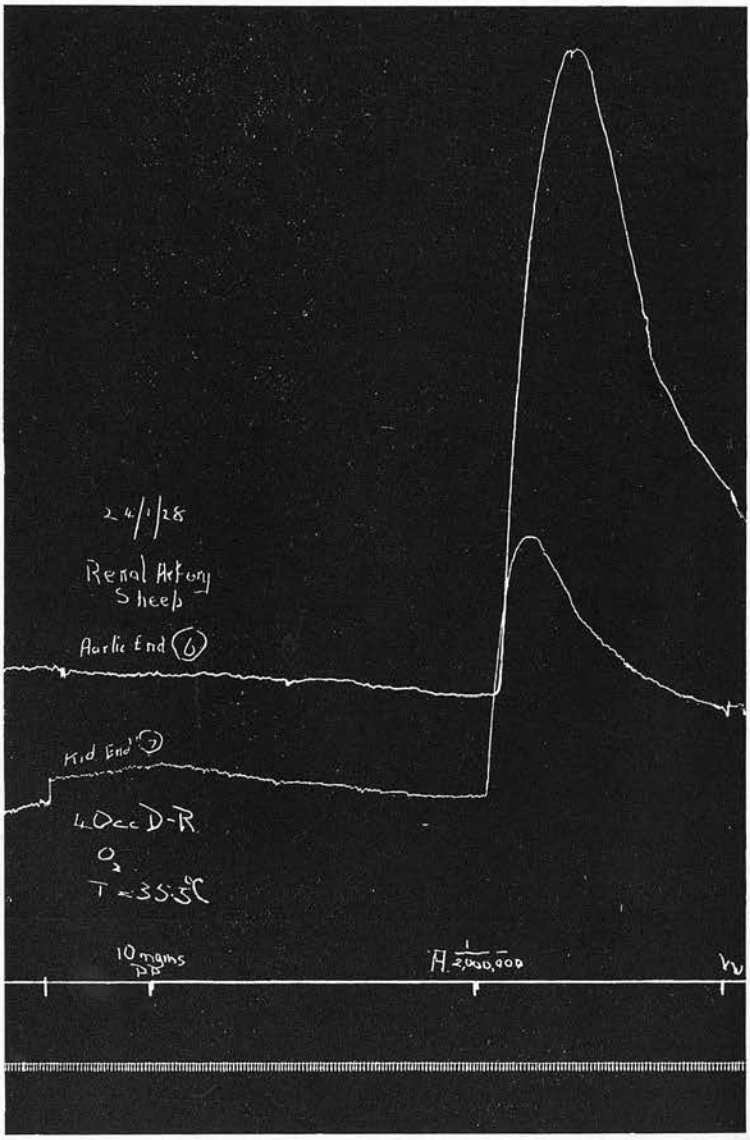


FIG. 2.—*Sheep-vessels.*—Tracing of the effects of extract of 10 mg. standard posterior lobe pituitary powder, and of adrenaline in a concentration of 1 in 2,000,000.
Upper record, 6 rings of renal artery (aortic end); lower record, 7 rings of renal artery (kidney end). Time in 10 seconds.

of the two ends of the renal artery, which, according to PAL (14), react differently. A slight relaxation is seen in the peripheral end, and no

response in the central end, with a concentration of 20 units in a 40 c.c. bath. The vessels are seen to be very sensitive to adrenaline.

In the 3 experiments on the renal vessels of the pig it was found that pituitary extracts produced marked contraction—2 units in 40 c.c. was equivalent to a concentration of about 1 in 1,000,000 adrenaline. In no other animal investigated were the large vessels markedly affected by the extracts, and, as stated above, slight contraction is seen rather more often than slight relaxation in the vessels to the kidney. The conclusion is that, except in the pig, in which the renal vessels are powerfully contracted by pituitary extracts, the large vessels, when isolated, do not respond by either relaxation or contraction to this drug, though easily affected by small doses of others.

It is realised that the part played in renal dilatation by the main artery or even its proximal branches is insignificant, but the smaller vessels cannot easily be studied in isolated preparations, and perfusion experiments produce constriction rather than dilatation.

Kidney Volume Changes, and their Relation to Diuresis.

In the earlier observations on the oncometric changes produced by pituitary extracts, the dilatation of the kidney, being the striking feature, received most of the attention. Several observers, however, have called attention to an initial constriction, and I have found this constriction preceding the dilatation in all good plethysmographic records in the present series. Sometimes it is very short, as in fig. 3. In other cases, though less prolonged, it may be as intense as the subsequent dilatation (see fig. 5).

Further, it has been found in extended experiments that the dilatation is succeeded by a slight diminution in volume which lasts for about half an hour or longer. To detect this secondary effect, it is necessary to have a well-fitting, well-sealed oncometer, and to leave the renal capsule *in situ*—decapsulation always produces some transudate of blood or lymph which causes a steady rise in the base-line, at any rate for some hours.

Two good examples of this secondary constriction are reproduced. Fig. 3 shows, in the record of kidney volume, a brief initial constriction, marked dilatation for 9 minutes, and a secondary constriction lasting for 21 minutes. This record is from an anæsthetised animal (chloralose), but similar changes are seen in a spinal preparation (fig. 4). This tracing is obtained 8 hours after the operation, so that the ether used for the pithing of the head would be thoroughly eliminated. Here the initial constriction lasts about 2 minutes, the dilatation 13 minutes, and the subsequent constriction 52 minutes. Thereafter blood-pressure and kidney volume are again normal. Although such secondary constrictions are less intense than the dilatations, they are more prolonged. In the light of this further evidence, the initial constriction, and the

constrictor effects reported by DALE (3) on the isolated perfused kidney, it seems not unlikely that constriction is the true response of the renal circulation to pituitary extract, and that the dilatation is passive, due to the expulsion of blood from other vessels which are more powerfully constricted into the less resistant circulation of the kidney. Once the

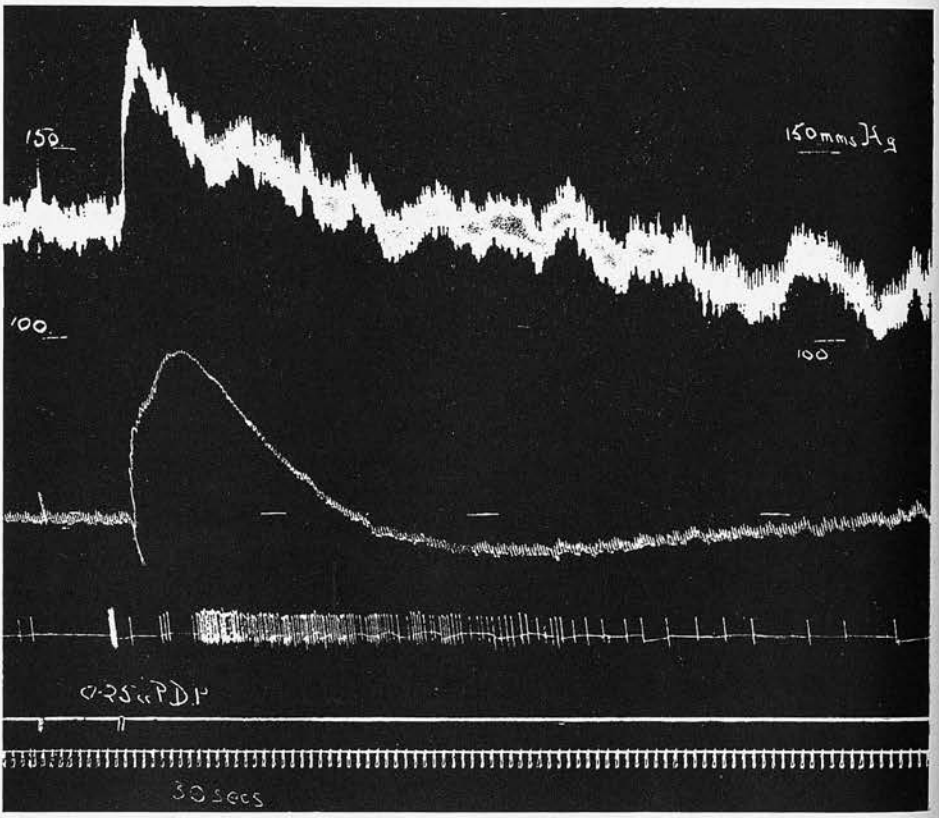


FIG. 3.—Record of the effects of 0·25 c.c. of Parke Davis pituitrin (depressor-free) on the blood-pressure, kidney volume, and secretion of urine in a chloralosed cat. Note that the renal dilatation, which duplicates the blood-pressure curve, is preceded and followed by constriction, and that the secretion of urine does not lag much behind.

body has had time to adjust itself to the effects of the vasoconstriction the dilatation can be dispensed with, and the kidney again constricts.

The duration of the constriction is interesting. It is common knowledge that after an injection of pituitary extract further doses give reduced effects on the blood-pressure, unless about an hour has elapsed, or even longer where large doses have been given, and this in spite of the fact that usually within 15 minutes the blood-pressure has resumed its usual level. If the vessels take about an hour to recover from the injection, as is suggested by the oncometric record in fig. 4,

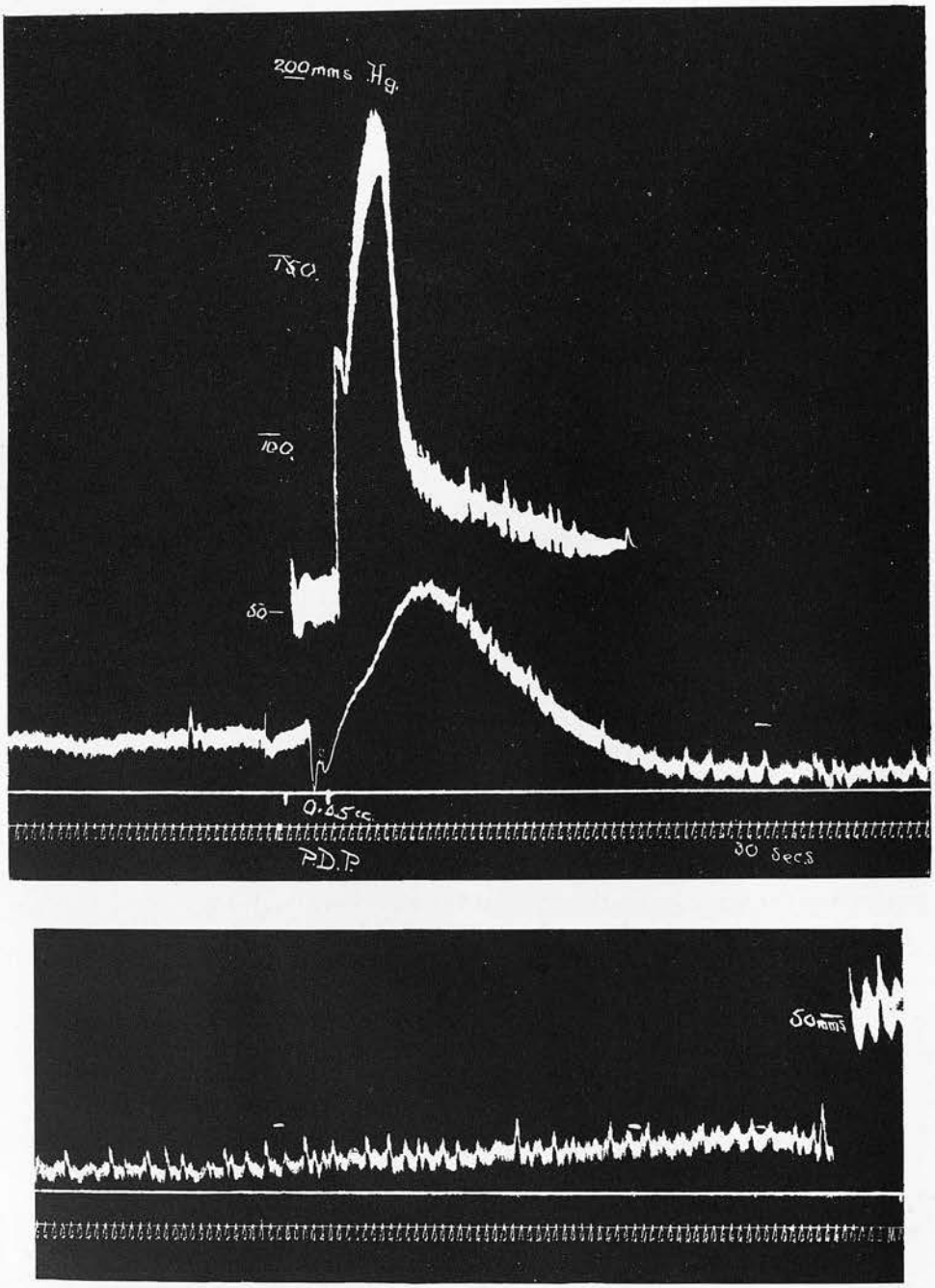


FIG. 4.—Record of kidney volume in a spinal cat, 8 hours after removal of the brain, showing the primary constriction, dilatation, and secondary constriction caused by pituitrin. In about an hour blood-pressure and kidney volume are again normal.

the "tachyphylaxis" can be readily understood. Similar prolonged effects have been obtained in records of limb volume. Quite clearly, the fact that the blood-pressure level has returned to normal is no guarantee that the vessels have completely recovered from the effects of the injection.

Can pituitary diuresis be ascribed to the circulatory changes? This has been denied in favour of a stimulation by the extract of secretory processes for two reasons:

(*a*) Because diuresis has been obtained without a rise of blood-pressure; and

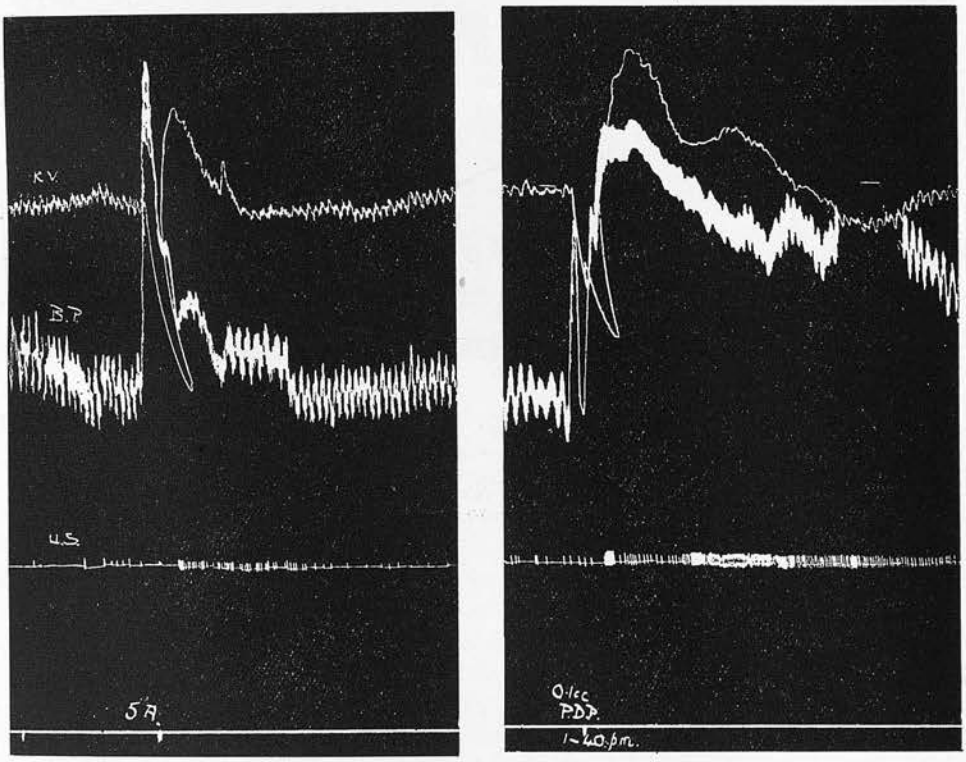
(*b*) because diuresis persists after the kidney dilatation has passed away.

Against this, in a large number of experiments performed in these laboratories, diuresis has never been detected without some accompanying dilatation and rise of blood-pressure, and as in figs. 3 and 5, the diuresis reaches a maximum shortly after the maximal renal dilatation, and does not outlast the dilatation by more than about 10 minutes. If the maximum dilatation corresponds with the maximal glomerular activity, while the urine-drops are drawn from the bladder, this 10-minute interval does not appear excessive. But further evidence in support of a circulatory origin of the diuresis is available.

Firstly, there is the similarity of the responses as regards blood-pressure, kidney volume, and secretion of urine provoked by other drugs which affect the circulation, to those seen with pituitary extract. Thus, in fig. 5, the responses are compared as produced by 0·01 mg. adrenaline hydrochloride and one unit of pituitrin. The adrenaline produces a much greater though more transitory rise of blood-pressure. Both drugs show dilatation of the kidney after an initial constriction, and a definitely increased secretion of urine. The differences between the responses are less striking than their similarities. Diuretic response to adrenaline is only obtained when a suitable dose is given, and is likely to be secondary to the circulatory changes rather than due to specific secretory stimulation.

Secondly, in the dog, as described by SHARPEY-SCHAFER and MACDONALD (18), the effects of pituitary extracts on the vessels are complicated by the effects on the heart. Where the vagi are intact and no atropine has been administered, cardiac inhibition is often striking. In such an animal, *e.g.* under chloralose, no diuresis is obtained; indeed anti-diuresis may be established at once. If the vagi be cut, and still more markedly if generous supplies of atropine be injected, the blood-pressure rises, the kidney dilates after an initial constriction, and the urinary excretion for the first fifteen minutes is more than doubled. One example of the two actions on the same animal is shown in fig. 6. The vagi have been divided, and several hours have elapsed in the interval between the tracings.

In the anæsthetised rabbit also it is not uncommon to get very little rise of blood-pressure and some cardiac embarrassment with intravenous administration of the extracts, and in such a case there is no increase of urine secretion, but a decrease. This occurs when the pressure is initially high, as in light anæsthesia, *e.g.* after chloralose. The responses in the rabbit seem to be intermediate to those found in

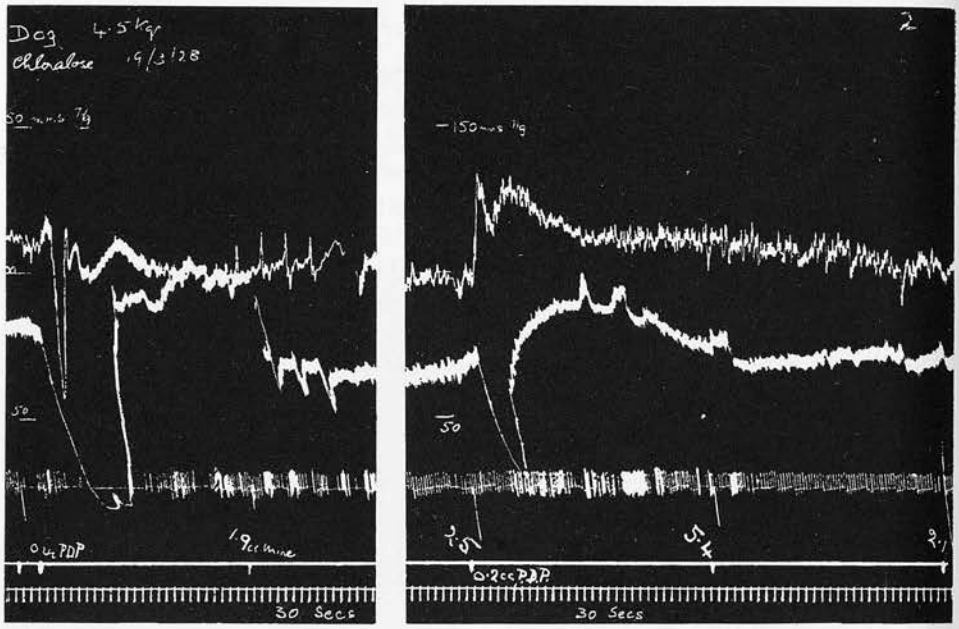


A B

FIG. 5.—These tracings compare the effects on blood-pressure, renal volume, and secretion of urine of 0·01 mg. adrenaline hydrochloride and 1 unit of pituitrin in a chloralosed cat. The pituitary effects are much more prolonged, but both show renal dilatation following on the constriction, associated with increase in urine secretion.

the cat and dog. (For records of the effects of pituitary injections on the circulation of the rabbit, see SHARPEY-SCHAFER and MACDONALD (18), figs. 12, 13, 14, 27, and 28.)

In studies of the rate of blood-flow through the kidney in the cat, rabbit, and dog during pituitary diuresis, several workers have obtained evidence of increased minute-volume. On ordinary intravenous injection in the cat I have found a slight initial reduction in the flow from the renal vein, and then a marked increase, corresponding respectively to the initial notch and succeeding dilatation in the plethysmographic record. If, however, traces of the drug be injected directly into the



A B

FIG. 6.—Comparison of the effects of 2 units of pituitrin on the blood-pressure, kidney volume, and secretion of urine in a dog under chloralose anæsthesia weighing 4·5 kilograms; before (A) and after (B) division of the vagi. In A, the vasoconstriction has so affected the heart that there is no rise in blood-pressure. In B, the preliminary well-marked kidney constriction is followed, with the rise in blood-pressure, by a dilatation, accompanied by marked increase in urinary secretion.

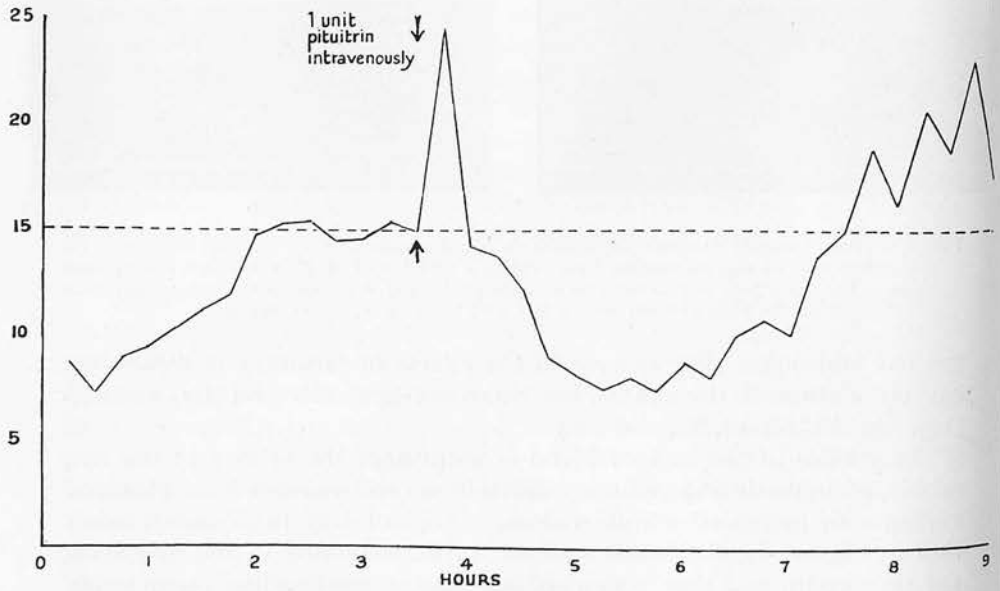


FIG. 7.—Record of secretion of urine measured every fifteen minutes for nine hours in a chloralosed cat, in which Ringer solution was infused throughout at a rate of 1 c.c. per minute—represented by the dotted line 15 c.c./15 minutes. The urine balances the infusion after 2 hours. At 3½ hours, 1 unit of pituitrin intravenously causes a marked diuresis in the ensuing ¼ hour, but thereafter there is marked anti-diuresis, lasting for the next 3½ hours, in spite of a constant infusion of Ringer.

renal artery, so that its first and main effects are on the kidney, this increased flow is not obtained, and the effects on the vessels and secretion are as in the perfused kidney. Unfortunately such blood-flow estimations are too "acute" to be satisfactory.

Further, the diuresis induced by pituitary extract has always been found to be followed, in some 20 experiments on cats and dogs, by a period in which the excretion is diminished. This anti-diuresis cannot be regarded as compensatory, due to the after-effects of increased secretory activity, for it is much too prolonged, and appears in animals receiving an infusion of Ringer solution at a constant rate, in which a constant compensating diuresis has been achieved. To carry an experiment on this point to a successful issue often takes about 10 or 12 hours, as the urine outflow lags well behind the input for a long time. A striking example of the short diuresis, long anti-diuresis, and recovery is shown in fig. 7, and explained in the legend to that graph.

Influence of Anæsthesia, and of Method of Administration of Extracts.

Since intravenous injection of extracts into the anæsthetised cat or rabbit causes diuresis, and subcutaneous administration to the non-anæsthetised animal or man has an anti-diuretic effect, the question arises as to whether it is the anæsthetic, or the sudden arrival of a fair concentration of the hormone in the circulation, such as occurs with intravenous injection, which determines the nature of the response. The work of Motzfeldt (13), and of Smith and McClosky (20), establishes that in unanæsthetised rabbits even with intravenous injections anti-diuresis is found when the secretion is measured at half-hourly or similar intervals. It will be realised from fig. 7 that hourly sampling may quite fail to detect a transient diuresis, but more frequent measurements are not without difficulty if disturbance of the animal is to be minimised. In man, so far as I am aware, no observations on the effects of *intravenous* administration on renal secretion have been published, so the following observations have been made.

In a number of cases attending a cystoscopic clinic, after indigo-carmine had been administered and its excretion detected by the cystoscope, the rate of emission of spurts of coloured urine from the ureteric orifice was observed before and after the intravenous administration of 2 units of pituitrin. The rate of appearance of the jets was definitely increased, but the volume of each spurt as indicated by the amount of dye entering the bladder appeared to be reduced, and this and the active appearance of the ureteric orifice suggested stimulation of the ureters. Such stimulation had been noted by Mackersie (11), who explained on this basis the initial check to secretion which often precedes diuresis in the experimental animal,

and which does not appear if a collecting catheter be inserted near the upper end of the ureter. It may be mentioned, incidentally, that although the human bladders were, as is usual for cystoscopy, comfortably filled, no complaint was made of painful spasm of the bladder, such as might have been expected from the observations of MACDONALD and MCCREA (10), who found marked stimulation of vesical contractions in the cat after pituitrin.

In two cases the average intervals between the spurts in seconds varied as follows:—

	Case I.	Case II.
Before pituitrin	47	23
30 seconds after 2 units, intravenously . .	10	14
1 minute later	4½	10
8 minutes later	8	12

Before one can feel satisfied about the apparent marked diuresis indicated by these figures, it is necessary to get some figures for the *volume* of the jets or the volume excreted per minute. In cases which a ureteral catheter was passed into the pelvis of the kidney (preparatory to pyelography), the minute volume excreted was noted before and after intravenous pituitrin.

The results were as follows:—

	Case III.	Case IV.	
	Urine per minute.	Urine per minute.	Blood-pressure average systolic. Mm. Hg.
Before pituitrin (average of 5 minutes).	0·23 c.c.	0·75 c.c.	145
After 2 units, intravenously:			
First 5 minutes . . .	0·16 ,,	0·82 ,,	138
Next 5 minutes . . .	0·16 ,,	0·81 ,,	135
,, ,, ,, . . .	0·15 ,,	0·67 ,,	135

No anæsthetic had been administered to these four cases, and no renal abnormality was detected in a most careful examination. With a ureteric catheter *in situ* there is no possibility of the ureter damming back the urine because of spasm. Urine might escape between the wall of the ureter and the catheter, but this is certainly unlikely after pituitrin. In the last case minute observations of systolic blood-pressure were taken, in case of marked changes. Probably diastolic figures would be more useful, but these, unfortunately, were not obtained.

In Case III. there is, from the beginning, a diminution in the secretion (30 per cent.). In Case IV. there is some slight (10 per cent.) but probably insignificant temporary increase in urine, with a slight fall in blood-pressure; but subsequent observations suggested that in this case the blood-pressure readings before the pituitrin administration had been raised by the examination and instrumentation.

It is desirable to extend these observations on man with observations of diastolic blood-pressure during the action of the drug, and possibly with the use of larger doses. It will also be interesting to note if in man after an anæsthetic, and possibly atropine, diuresis can be induced.

Discussion and Conclusions.

The weight of evidence in favour of the anti-diuretic action of pituitary extracts on renal secretion as the real response is overwhelming; it is seen alike in the perfused kidney, in man, and in the experimental animal. Even when there is diuresis such as occurs under favourable conditions, this is followed by prolonged anti-diuresis.

The explanation of the diuretic effect is controversial. As to whether or not it is secondary to circulatory changes, the literature chronicles a series of contradictory opinions which are extremely difficult to reconcile, but undoubtedly many of the workers obscured the true actions by the use of unpurified preparations containing depressor substances.

In the present experiments and in the more recent work on the subject (2, 20), in which depressor-free extracts have been used, the diuretic effects are transitory and are followed by anti-diuresis. To ascribe the diuretic effects, with Smith and McClosky, to the action of the anæsthetic is scarcely reasonable, since (*a*) in the anæsthetised dog and rabbit diuresis may not be produced at all, and (*b*) diuresis can be produced in the spinal cat. In these animals, diuresis occurs if the administration of the drug produces a significant rise in blood-pressure. It has not been sufficiently realised that in the unanæsthetised animal or in the lightly anæsthetised dog or rabbit (*e.g.* after chloralose) with the blood-pressure fairly high and the circulation in good condition, pituitary extracts, even intravenously, cause very little rise in blood-pressure, if any. Where the animal has received chloroform, ether, morphine, urethane, or the like, and very often a combination of depressants plus atropine, the blood-pressure is usually raised by pituitary extracts and diuresis follows. Observations on the rate of blood-flow through the kidney, and plethysmographic studies alike favour a circulatory explanation of the diuresis.

It is a pleasure to make grateful acknowledgment to Mr E. D. Portman for assistance in the earlier animal experiments, and to

Mr E. D. McCrea, of Salford Royal Hospital, for facilities and help in the experiments on man.

Summary.

1. The action of posterior lobe pituitary extracts on the renal vessels is not dilator, as is usually stated. In the pig, marked contraction has been found. In the cat, rabbit, sheep, and cow the plain muscle of the arteries is scarcely affected by pituitary extracts, though sensitive to other drugs.

2. The full course of the action of the extracts on the volume of the kidney *in situ* in the cat, whether anæsthetised or spinal, and, under favourable circumstances, in the dog, consists of a brief initial diminution in volume, a short increase in volume, and a prolonged secondary diminution. The increase in volume is believed to be passive; it can be produced also by adrenaline.

3. It is shown that in unanæsthetised man and in the lightly anæsthetised dog even intravenous injection may not raise the blood-pressure nor increase excretion. Diuresis can be produced in the decerebrate or spinal cat, and is thus less dependent on the presence of an anæsthetic than on the action taking place in an animal in which the blood-pressure can be significantly raised, as in the cat or in the dog after ether or morphine and atropine.

4. It is concluded from experiments on the cat, dog, rabbit, and man that the diuretic action is associated with rise of blood-pressure, and is always followed by anti-diuresis.

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Actions of posterior pituitary principles on the colon.

By A. D. Macdonald and H. L. Settle.

Since Blair Bell's [1909] observations on the stimulation of peristalsis in the rabbit and in man by pituitary extracts, widely varying results have been reported from similar experiments. Since the separation of the pressor and oxytocic principles in 1928 the position has become more complicated. Gruber and Robinson [1929] found both fractions inactive on the ileum of the dog, and we similarly got either no response or an inhibitory one on the colon of the cat. Elmer and Ptaszek [1930] concluded that in man "pitressin" was a more potent stimulant than "pituitrin", and, from experiments on rabbits and dogs, that "pitocin" antagonized the "pitressin" effect, thus explaining the pitressin-pituitrin discrepancy. Carlson [1930] confirmed the differences between the responses of man and the dog to the extracts, using three patients with colostomies for his human experimental material.

We have used the separated principles and combinations of them on a series of fifteen patients with colostomies, recording the movements of the proximal loop of the colon—believed healthy (one colostomy was of 8 years' standing)—by the usual balloon technique. The findings may be tabulated as follows:

	Pitressin (1 or 2 units)	Pitocin (5 or 10 units)	Pitressin (1 unit) + Pitocin (5 units)
Number of injections	15	11	3
Stimulation	15	3	3
No action	0	8	0

In these experiments the drugs were injected intravenously—the responses to subcutaneous or intramuscular administration were often feeble and always delayed. Stimulation as recorded on the drum, 2–3 min. after injection, was usually accompanied by the loss of fæces or flatus. The table indicates that small intravenous doses of pitressin are peristalsis-stimulating in man, and that pitocin in larger doses does not inhibit this response. The occasional response to pitocin alone may be due to a sensitive muscle being affected by the persistence after separation of a trace of pitressin (5 p.c.). The records have had further support from screening observations on three patients after opaque meals.

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THE PHARMACOLOGY OF PERCAINE

BY

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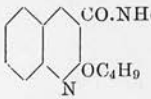
While considerable clinical experience of percaine has been reported in this country, in the *British Medical Journal* and elsewhere, both as a local anaesthetic (Lake and Marshall,[1] Walker,[2] Popper[3]) and for spinal anaesthesia (Howard Jones[4]), no report of the pharmacology of this drug has, to our knowledge, appeared from a British laboratory. In view of the discrepant findings of Continental workers (Uhlmann,[5] Lipschitz and Laubender[6 7]), and of the increasing use of local and regional anaesthesia in surgery, a further investigation of percaine, and a comparison of it with the more established members of the group, seemed desirable.

Percaine is a quinoline derivation—the hydrochloride of the diethylethylenediamide of α-butyloxycinchoninic acid.



It is manufactured at Basle, and the first publication of its properties was made in June, 1929, by Uhlmann.[5] It is available in crystalline form, and is colourless, odourless, and easily soluble in water or normal saline, but is readily precipitated by alkali. The makers' claims—that it can be heat-sterilized without loss of strength, and that it can be combined with adrenaline—have been confirmed. Sterile solutions are stable, retaining full activity for several months at least. We have had some evidence that solutions which have not been kept under aseptic conditions lose their activity in a relatively short time.

The following experimental investigations have been carried out, in the hope that the value of the new drug might be fairly assessed.

Intradermal Wheal Test on the Human Subject

Solutions of various strengths in 0.9 per cent. NaCl were injected intradermally, always using 0.5 c.cm. for a test. The sensation of pain was tested by the application to the skin of a pin-point suitably mounted. Tests were directed to determine (*a*) the minimal concentration required for anaesthesia as compared with novocain, and (*b*) the duration of the anaesthesia afforded by any given concentration. The results are given in tabular form, showing (1) the drugs used by themselves, and (2) with adrenaline as an adjuvant. It will be seen (1) that the minimal effective concentration of percaine is about one-fortieth of that of novocain ; (2) that 0.05 per cent. percaine produces anaesthesia for longer than 1 per cent.

TABLE I

Drug	Concentration (per cent.)	Duration of Anaesthesia in Minutes	
		(1) Without adrenaline	(2) With addition of adrenaline, 0.002 per cent.
Percaine ..	0.008	0	(60)
	0.012	0	100
	0.016	0	240
	0.025	35	280
	0.05	70	320
Novocain ...	0.12	0	(60)
	0.25	0	160
	0.50	6	220
	1.00	25	270

Note.—Adrenaline alone is probably responsible for the anaesthesia shown in parenthesis.

novocain ; (3) that when both are mixed with adrenaline 0.002 per cent., 0.05 per cent. percaine and 1 per cent. novocain produce anaesthesia of equal duration in the skin—nearly five hours.

Blocking of the Nervous Impulse in the Frog's Nerve-Muscle Preparation

This test is mainly of academic interest, being valuable for the comparison of the ability of anaesthetics to block

impulses in motor nerve fibres. About 1 cm. of the nerve of the ordinary sciatic-gastrocnemius preparation is immersed in a solution of the anaesthetic, and the duration of immersion in which conduction is just suspended is determined. The two hind limbs of the frog yield two similar preparations, so that two drugs can be fairly accurately compared for their activity. By this test percaine is about seven times as efficacious as cocaine.

Anaesthesia of the Rabbit's Cornea

This test is of importance for its application to ophthalmological practice in particular, and the anaesthesia of mucous membranes in general. The method prescribed by Sollman[8] was used. The conjunctival sac

TABLE II.—*Anaesthesia of the Rabbit's Cornea*

Percaine

Dilution (per cent.) ...	0.002	0.005	0.010	0.017	0.025	0.05
Duration (minutes) ...	0	0	10–15	45–60	90–120	over 180

Cocaine

Dilution (per cent.) ...	0.2	0.5	1.0	2.0
Duration (minutes)	0	7–10	15– 0	30 40

is flooded with the solution under test for one minute, and the corneal reflex is stimulated at intervals until it returns. The durations of anaesthesia thus obtained for various concentrations of percaine and cocaine are given in Table II.

It will be evident that percaine is efficacious in about one-fiftieth of the concentration of cocaine which abolishes the reflex. No adrenaline was added to the solutions in any of these experiments.

Experimental Spinal Block in the Cat

In two of three experiments in which novocain and percaine were alternately introduced by lumbar puncture into the spinal theca of a chloralosed cat, it was found that percaine abolished active reflexes, such as the knee-jerk, in a dosage of about one-fortieth of that of novocain, and the anaesthesia, as tested by the abolition of these reflexes, lasted three or four times as long—for example, two hours with 0.5 mg. of percaine, as against half an hour with 20 mg. novocain. This is quite in accordance with the claims made for percaine by Howard Jones.[4]

Toxicity of Percaine

This is obviously a matter of great importance, for percaine, like other local anaesthetics, falls short of the ideal in being a distinctly toxic drug. We have carried out estimates of relative toxicities by the lethal dose following (1) subcutaneous injection into guinea-pigs, and (2) intravenous injection into cats, using paralysis of respiration as an end-point. The details of this second method, which we have elaborated in order to get a more accurate measurement of relative toxicity—two drugs being compared on a single animal—will be published elsewhere.[9]

The results of the guinea-pig method indicate that percaine is about twenty-five times as toxic as novocain and three times as toxic as cocaine ; by the cat method the corresponding figures are fifteen and two respectively, and in the authors' opinion the latter figures are the more reliable. Taking these figures we can form, as in Table III, an estimate of the relative efficiencies of the three drugs.

TABLE III.—*Relative Efficiency*

Relative Efficiency	Percaine	Cocaine	Novocain
(*a*) In intradermal wheal	10	1	6
(*b*) In rabbit's cornea	25	1	$\frac{1}{3}$ or less
(*c*) As spinal anaesthetic	3	—	1

The efficiency of a local anaesthetic for any purpose is determined by the ratio of the active or adequate dose to the toxic dose.

DISCUSSION

The figures of Table III suggest that percaine and novocain are of the same order of efficiency when used for subcutaneous anaesthesia, and the difference in favour of percaine for spinal anaesthesia is probably not as great as it appears to be, for the action of novocain intrathecally can be limited and intensified and prolonged by giving it in a viscous solution. On the other hand, for local application to mucous membranes percaine is quite the most efficient local anaesthetic we have so far investigated. As far as can be seen, no injurious results follow from continued application of the drug to mucous membranes—even to a membrane as delicate as the conjunctiva. Thus 0.05 per cent. percaine was applied once a day for fourteen days to the eye of a rabbit without any noticeable ill effects. The fact, noted by Uhlmann,[5] that much higher concentrations may cause

corneal opacities is of toxicological rather than of ordinary practical interest.

From these experiments we conclude that for infiltration anaesthesia, in view of its considerably greater toxicity, no great advantage can be claimed for percaine as a substitute for the novocain-adrenaline combination to which the surgeon is well accustomed. Occasionally, where adrenaline action is to be avoided, percaine would supply an adequate duration of anaesthesia without interfering significantly with the local circulation ; and percaine is a cheap substitute for novocain, especially since it is effective in considerable dilution. For mucous membranes, on the other hand, we agree with Popper[3] that percaine is a most promising addition to the surgeon's pharmacopoeia, and may well pave the way to the abandonment of cocaine.

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THE ESTIMATION OF THE RELATIVE TOXICITIES AND EFFICIENCIES OF LOCAL ANESTHETICS

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1. THE DETERMINATION OF RELATIVE TOXICITIES OF LOCAL ANESTHETICS

The perfect local anesthetic would be a non-toxic substance, but, since such an ideal has not yet been discovered, it is essential, for the estimation of the efficiency of a drug as a local anesthetic, that its toxicity, relative to that of the other members of the series, should be taken into account.

In the past various animals and techniques have been used in estimating the toxicity of local anesthetics, and there has been much discussion as to the most suitable animal to use, the method of administration, and the means of judging the final result. Thus Closson (1914) injected the drugs subcutaneously into guinea pigs; Eggleston and Hatcher (1919) used cats and injected the whole dose intravenously in five to fifteen seconds; Sollman (1920) immersed tadpoles and earthworms in solutions of the drug; Meeker and Frazer (1924) utilized the ear vein of the rabbit; Tatum *et al.* (1925) and Knoeffel *et al.* (1930) estimated the M.L.D. on the basis of subcutaneous injections into rabbits; Trevan (1927) recommends the intravenous injection of mice; more recently Rider (1930) proposes to use white mice and white rats injected subcutaneously. Inevitably, no one author's figures are comparable with another's, and the main impressions one gathers from the literature are that animals vary extraordinarily in their resistance to the toxic action of such drugs and that large numbers of animals must be sacrificed if any accurate measure of the M.L.D. is to be obtained. Trevan's estimate of 30 as an opti-

for an unknown drug, can only be determined by trial and error. The same is true for the determination of the dilution of the anesthetic which is desirable, but usually the first infusion will suffice to give a fair estimate of the concentration required.

Every infusion of local anesthetic would give an estimation of the lethal dose, but the use of the method lies not so much in determining the individual lethal dose on a number of cats for a particular drug, as in the opportunity it provides for comparing one drug with another, as regards toxicity, *on the same animal and under very similar conditions*, just as two pituitary extracts may be compared on a single cat for pressor activity, or a single guinea pig uterus for oxytocic titer. The absolute M.L.D. is found to vary from cat to cat through wide ranges for no obvious reason. It also varies in any one cat according to the rate of administration of the drug. This is to be expected since the concentration of drug in any tissue is a function not only of the rate of administration but also of the rates of destruction and elimination. Some justification of the method as an assay method, and the evidence for its dependence on a constant rate of administration, will be gleaned from the two experiments summarized in table 1, in which novocaine is infused at varying rates with one hour intervals between doses.

Both experiments include one observation in which the drug was infused too slowly, and another in which it was supplied too rapidly, with resultant discrepancies in the lethal dose. Both show very fair agreement in the results obtainable with comparable rates of administration.

A tracing of a part of an experiment will help to make the method clear (fig. 1). Three infusions are shown. In the first novocaine is run in fast enough (but at a constant rate, 1.5 cc. per minute) to paralyze respiration in seventeen minutes. Believing that β-eucaine was about equally as toxic as novocaine, a solution of the same strength was run in at the same rate one hour later but produced respiratory paralysis in ten minutes. Either the novocaine effect had not worn off, or β-eucaine would seem to be more toxic to cats. A later infusion at a slower rate (fig. 1, *c*, 1.1 cc.

per minute) proved the second possibility to be actually true, as did another experiment.

A conceivable objection to the method is that we are allowing the animal to destroy or eliminate the drug while the infusion is going on, and the figures obtained are accordingly inaccurate. But in clinical poisoning with local anesthetics there is similarly slow absorption and accumulation—probably slower since in our experiments we are administering the drug by direct intravenous

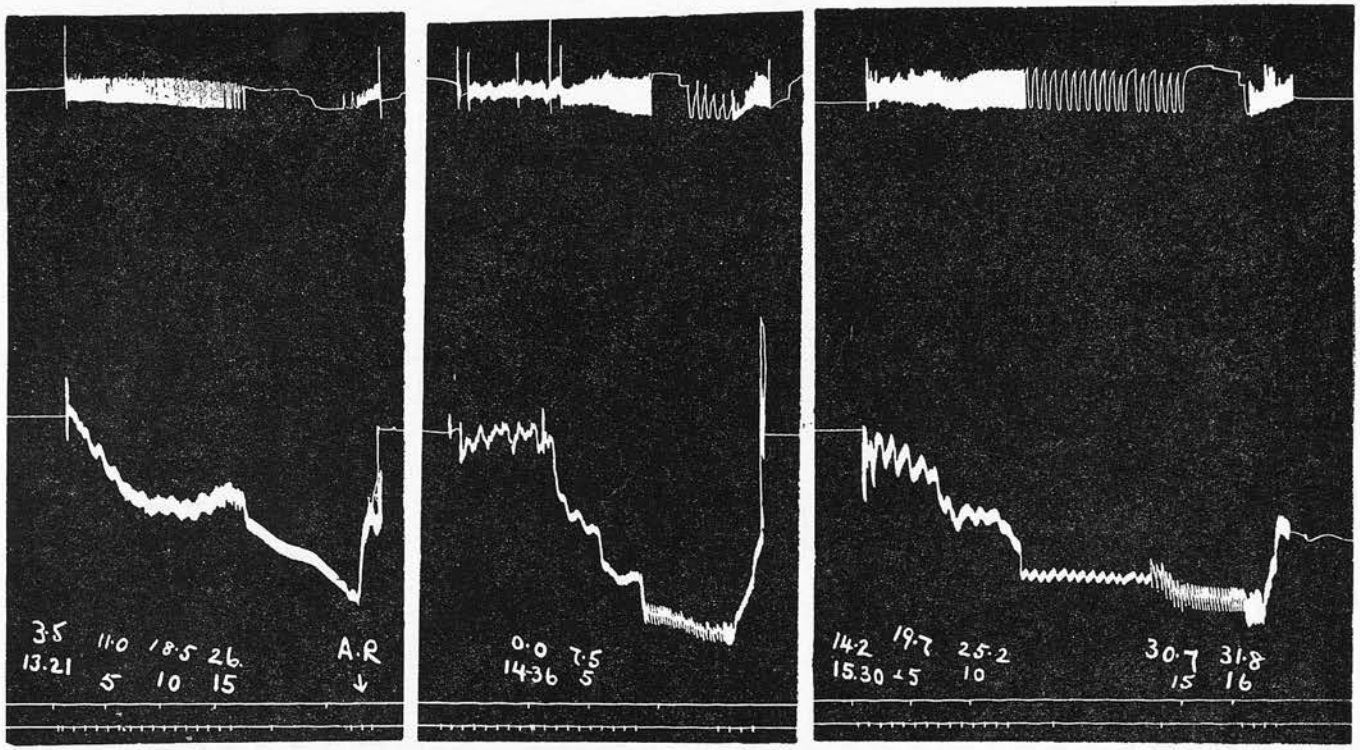
TABLE 1

RATE OF ADMINISTRATION OF NOVOCAINE	TIME TO PARALYZE RESPIRATION	DOSE WHICH PARALYZES RESPIRATION
Cat 7, April 17, 1931, 2,520 grams, dial, 1.3 cc.		
mgm. per kgm. per minute	*minutes*	*mgm. per kgm.*
2.43	15	36.5
1.51	36	54.3
1.66	33	54.8
1.43	49	70.5
1.91	28	53.5
Cat 12, May 6, 1931, 2,800 grams, dial, 1.5 cc.		
2.12	28	62.4
2.62	26	68.1
2.96	9	26.6
2.84	25	73.5
1.85	Not in 60	Over 112
2.62	28	73.6

injection. An important clinical exception to this occurs in spinal anesthesia where a high concentration of local anesthetic, introduced by lumbar puncture, may act at times directly on the respiratory center, and act with alarming speed and effect, but this is a special and rare case.

We have estimated the relative toxicities of a number of widely used local anesthetics by this method, and the results are given in table 2.

Certain points which cannot readily be represented in such a table invite comment. In the first place, the rates of elimination of the drugs differ. Thus after respiratory paralysis due to novo-



a *b* *c*

FIG. 1. TRACINGS OF RESPIRATION AND BLOOD PRESSURE DURING THREE INFUSIONS OF LOCAL ANESTHETIC INTO A CAT UNDER "LIQUID DIAL"

Time tracings show minutes, the kymograph being "geared-up" for the end-points. *A. R.*, artificial respiration.

a. Infusion of novocaine. Respiration is paralyzed in seventeen minutes by running in 1.5 cc. of solution per minute, equivalent to 34.2 mgm. per kilogram body weight.

b. Interval of sixty minutes. Infusion of β-eucaine. Respiration is paralyzed in ten minutes by running in 1.5 cc. of solution per minute, equivalent to 19 mgm. per body weight.

c. Interval of ninety minutes. Infusion of β-eucaine. Respiration is paralyzed in sixteen minutes by running in 1.1 cc. per minute equivalent to 23.8 mgm. per kilogram body weight.

The rate of infusion is rather fast in all three experiments, especially in *b*, but *a* and *c* are comparable and indicate that β-eucaine is about one and one-half times as toxic as novocaine. Note the prompt recovery of the blood pressure when artificial respiration is supplied.

caine infusion, it is sufficient to allow an hour's interval, with twenty minutes or half an hour of artificial respiration, before a subsequent infusion. With percaine and stovaine, about two hours are required. β-Eucaine is intermediate. Cocaine differs from the others, in our experience, in that after respiratory paralysis following cocainization, even although natural respiration is resumed in about an hour, the animal remains sensitive to the action of further infusions of local anesthetics, whether cocaine or novocaine, for many hours, and is no longer of value for assay. Thus in comparing other drugs with cocaine, the cocaine infusion should be terminal. This would seem to afford further evidence that the destruction of elimination of cocaine must be relatively

TABLE 2

DRUG HYDROCHLORIDE OF	RELATIVE TOXICITIES	
	Method of respiratory paralysis (cat)	Subcutaneous injection of guinea pig
Novocaine	1	1
Percaine	12	23
Stovaine	1	2
β-eucaine	1.5	1
Cocaine	6	8

slow, or, alternatively, that cocaine "sensitizes" the respiratory center in some way to the action of local anesthetics. The latter possibility is improbable in the light of Eggleston and Hatcher's (1919) criticism of Grode (1912), who thought he had evidence of increased susceptibility to cocaine in animals injected daily with the drug, whereas actually elimination, fixation or destruction of cocaine may take up to two to three days. Similarly, in Mills' (1919) experiments the effects of cocaine on the eye of the rabbit persisted for about twenty-five hours. This method of assay allows a relatively accurate estimation of the rate of destruction of the other cocaine substitutes. If the minimum rest interval be determined for which equal doses are required to paralyze respiration, that interval gives, presumably, the time required for the destruction of the given amount.

In the second place, Eggleston and Hatcher found that, for the resuscitation of animals poisoned with massive doses of local anesthetics, given intravenously, artificial respiration, cardiac massage, and intravenous adrenaline were all necessary. When the drug is administered gradually, as in our experiments, artificial respiration alone is unfailingly successful, and hence presumably in man when it is also absorbed gradually, artificial respiration is much the most important part of the treatment, and may be expected to succeed, if begun in time, without cardiac massage and intravenous adrenaline.

Further, it will be noticed in table 2 that the relative toxicity figures obtained by the method of respiratory paralysis in cats differ from those given in the literature by the method of lethal dosage in guinea pigs.

We feel sure that our figures, obtained from experiments in which the drugs are compared on a single indicator, and therefore free from many of the objections to which biological assays are open, are the more reliable. They will not, of course, give any indication of the relative incidence of minor toxic symptoms, such as headache or emesis, but only clinical experience can do this.

The method we have described may demand a little more skill and time than the injection of a group of mice and the subsequent enumeration of the dead, but it makes no excessive demands on time, patience, or skill, and can be readily carried out in any laboratory equipped for animal experimentation.

2. DETERMINATION OF THE RELATIVE EFFICIENCIES OF LOCAL ANESTHETICS

The "efficiency" of a local anesthetic, as with other drugs, is measured by the ratio of the minimal lethal dose to the lowest adequate concentration, M.L.D./L.A.C. To the use of this ratio as a criterion of clinical efficiency the objection may be put forward that only rarely will such drugs be used in amounts approaching those producing toxic symptoms; but without some such criterion it is difficult to take into account the toxicity factor, which is admittedly important in chemotherapy. To omit such a consid-

eration after allowing its importance "since it does not serve as an assay method" as in Munch's recent book on "Bio-Assays" (1931), seems to the present writers to be both unfortunate and unjustifiable.

However, the actual value of the "efficiency" of any given local anesthetic is not of so great importance as its relative efficiency compared with other drugs of this type. This relative efficiency can be computed without knowing the actual M.L.D.'s or L.A.C.'s of other drugs if the "relative efficacy"—i.e., the ratio of L.A.C.'s—and the relative toxicity be known.

$$\text{Then relative efficiency} = \frac{\text{Relative efficacy}}{\text{Relative toxicity}}$$

TABLE 3

DRUG	RELATIVE TOXICITY	RELATIVE EFFICACY		RELATIVE EFFICIENCY	
		Intradermal wheal	Rabbit cornea	Intradermal wheal	Rabbit cornea
Cocaine	100 (6)	100	100	1	1
Novocaine	17 (1)	100	6	6	$\frac{1}{3}$
β-eucaine	29 (1.5)	50	50	2	2
Percaine	200 (12)	2,000	5,000	10	25

The figures in parentheses in the relative toxicity column show the toxicity figures with novocaine = 1. The other figures have been adjusted so as to give the efficiencies of cocaine as unity.

The advantage of the method described for the determination of the relative toxicity is that it enables this important factor to be assessed rapidly without determining the actual M.L.D.'s of any of the drugs, which figures, though no doubt of great interest, are not essential for arriving at an estimate of the value of any one local anesthetic as compared with others.

With regard to the relative efficacy, it is the writers' belief that, whatever else may be desirable or interesting, the relative efficacy under two conditions must be known: (1) in the intradermal wheal on the human subject; (2) on the rabbit's cornea.

From these considerations the relative efficiency of any given local anesthetic drug may be arrived at under conditions that

will give some indication of their relative clinical efficiency (1) for infiltration anesthesia, and (2) for the anesthesia of mucous membranes. Table 3 shows these relations for some of the better known local anesthetics as compared with percaine, the pharmacology of which we have been re-investigating and have reported elsewhere (Israëls and Macdonald (1931)).

SUMMARY

1. A method is described by which measures of toxicity, relative toxicity, and rate of destruction of local anesthetics can be obtained following slow intravenous infusion into cats.

Figures for the relative toxicities of common local anesthetics are given, and some of the applications of these experiments to the clinical use of these drugs are discussed.

2. A fair indication of the relative efficiencies of local anesthetics can be obtained in terms of their relative efficacies (ratios of concentrations which achieve adequate anesthesia for any purpose) and relative toxicities.

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OBSERVATIONS ON EXPERIMENTAL SPINAL ANESTHESIA[1]

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INTRODUCTION

These experiments were undertaken with the object of investigating what rôle, if any, the anesthetic plays in the occasional fatalities which occur in surgical operations in which a spinal anesthetic has been used. Spinal anesthesia, since its introduction, has had periods of popularity and of unpopularity. Today, for many surgeons, it is wherever possible the method of choice. We do not wish to discuss the pros and cons of this attitude, but clinical journals periodically report deaths on the table and speculate on the etiology of such an issue. One of us had three such fatalities (Hill, 1931). "We need" says Roeder in a recent paper, "an accurate explanation of the vascular and respiratory collapse following the injection of anesthetizing substances into the subarachnoid space." Experimental investigations designed to afford such an explanation are few. "La rachianesthesie," according to Leriche (1928), "n'a pas eu encore l'heureuse fortune d'interesser les physiologistes." This is all the more surprising since every case of spinal anesthesia provides so much of interest to the physiologically-minded, and since the method, which presents no serious difficulty in the experimental

[1] Since completing this paper, our attention has been directed to a paper on experimental spinal anesthesia in dogs by Ferguson and North (Surg., Gynecol. and Obstet., April, 1932, liv, 4). We are surprised to hear that intrathecal injection in the dog presents difficulties which we have not met with in the smaller animal. The circulatory disturbances of spinal anesthesia in the non-anesthetized dog are apparently more serious than those we have encountered in the cat, but we can confirm the main findings in this paper from our observations.

animal, supplies a convenient method of temporary decentralization for hind limbs, viscera, and so on.

MATERIALS AND METHODS

Our experiments have been limited to some 50 cats, and these have been lightly anesthetized with "liquid dial" intraperitoneally —0.5 cc. per kilogram body weight, or, after a little ether, with an intravenous injection of a solution of chloralose, 80 mgm. per kilogram. On the whole the latter has proved more suitable. Lumbar puncture with a No. 16 hypodermic needle is carried out in the midline of the spine under the fourth or fifth lumbar vertebra. Though the lower space is the larger it seems, in practice, easier to insert the needle between the fourth and fifth. We have also carried out a number of experiments in which drugs have been injected directly into the cisterna magna. For this purpose a similar needle, fitted with a cross-bar 10 mm. from its point, is inserted into the cisterna between the atlas and the occipital bone. The cross-bar prevents the needle from penetrating too deeply, and a stitch around it maintains it in position. For insertion, both needles are fitted with stilettes, and, unless a free flow of cerebrospinal fluid is obtained on withdrawing the stillette, the needle is withdrawn and re-inserted. The cat is then placed on its left side, and the usual arrangements are made for recording blood pressure from the right carotid artery, and respiration from the costal margin or abdominal wall. A tracheal cannula is inserted so that artificial respiration can be given when necessary.

The drugs examined, such as novocain, stovain and percain, have been injected dissolved in saline at body temperature, and usually the amount of an injection has been limited to 0.5 cc. The mere mechanical effects of such injections have been controlled by comparison with injections of isotonic salt solutions. Usually 0.5 cc. of saline can be injected either by the lumbar route or intracisternally without significant effect on respiration or blood pressure (fig. 2, *A*, and 3). When we have used proprietory anesthetic solutions, such as "spinocain" or "duracain," we have diluted them with cerebrospinal fluid, as is practised in man.

In certain experiments we have endeavoured to limit the action of the drug to the spinal cord by removing the spinous process and part of the body of the axis, and opening the membranes to expose the cord. In other cases, by a similar operation on the seventh cervical or first dorsal vertebra, we have studied the effects of lumbar injections where the drug cannot so easily extend beyond the lumbar and dorsal cord. Even after the provision of such openings, however, we have found evidence indicating that the anesthetic has penetrated to higher levels when any considerable bulk of solution is used, unless it be injected very slowly. In other cases we have cut the cord at similar levels before using the anesthetic, to ensure its limitation to the desired area.

RESULTS

1. The action of novocain, injected into the cisterna magna. Injections of 5 or 10 mgm. in 0.5 cc. saline produce prompt paralysis of the respiration from direct action on the center. The blood pressure, in a lightly anesthetized cat (fig. 1), first rises—often to a remarkable level—and this rise is not asphyxial, for it appears before the respiratory center is overwhelmed, as well as in animals supplied with adequate artificial respiration throughout. With the development of respiratory paralysis, the blood pressure falls, but positive ventilation rapidly restores it, often to a very high level. Spontaneous respiratory movements usually occur in seven or eight minutes, and artificial respiration can then be stopped. With the bigger doses, recovery is slower, but, provided ventilation is maintained, very large and repeated doses are non-fatal.

The rise in blood pressure following intracisternal injections has been described by Dixon. It is not in any sense a reaction specific to novocain, and is explained by him as due to stimulation of a center controlling adrenaline secretion. We can confirm this explanation, for the rise is abolished by ergotamine or adrenalectomy. Further, in animals deeply anesthetized, or recently given ether, in which adrenaline is depressant to blood pressure, the rise scarcely occurs, and the collapse of blood pressure is unusually rapid (fig. 2). This variation in the results of stimulating

a center controlling adrenaline secretion is to be expected from the observations of Macdonald and Schlapp (1926), who have emphasized the importance of the anesthesia in adrenaline vasodilatation.

In one experiment, which we have failed to repeat in spite of several attempts to duplicate the conditions, the fall of blood

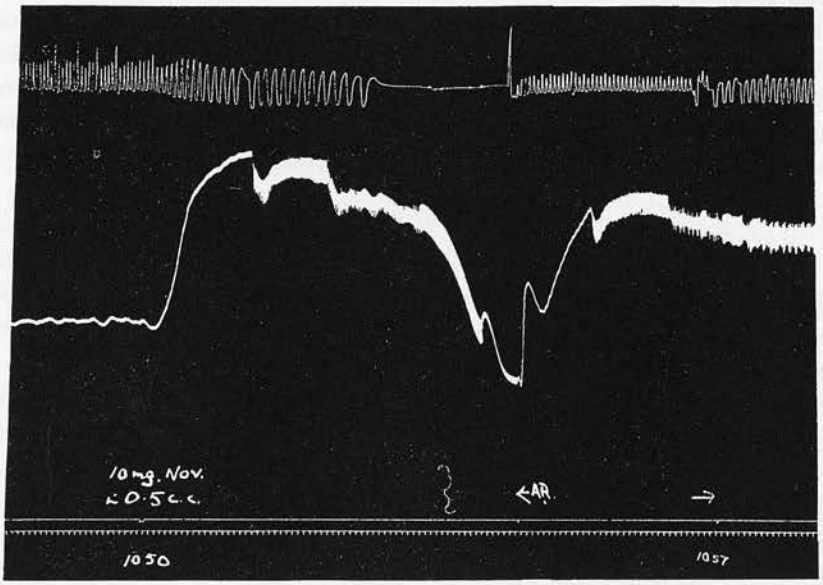

FIG. 1. ♂, 2040 grams, March 10, 1932. Chloralose. Note effects on respiration and blood pressure of an intracisternal injection of 10 mgm. novocain HCl in 0.5 cc. saline. Respiration fails in the third minute, but after two minutes artificial respiration (A. R.) the respiratory center recovers. With the large rise in blood pressure the heart is slowed.

(In this and subsequent tracings, the signal line represents zero blood pressure, and the time tracings show intervals of five seconds.)

pressure associated with failure of respiration was accompanied by marked cardiac slowing, suggesting central stimulation of the vagus, or heart block. When the blood pressure has been reduced for some time, before artificial respiration is supplied, heart block is common, presumably because of the ischemia, but our experience, with the exception of this one experiment, is that the heart is unaffected till late in the respiratory failure. With

large and repeated injections of the drug, it is possible to depress the vasomotor center but only with doses which far exceed the proportional maximal dose used in man. Thus the dose in man, injected by the lumbar route and in a viscous solution, rarely exceeds 300 mgm. The corresponding dose for a cat would be of the order of 10 mgm., and double this amount whether adminis-

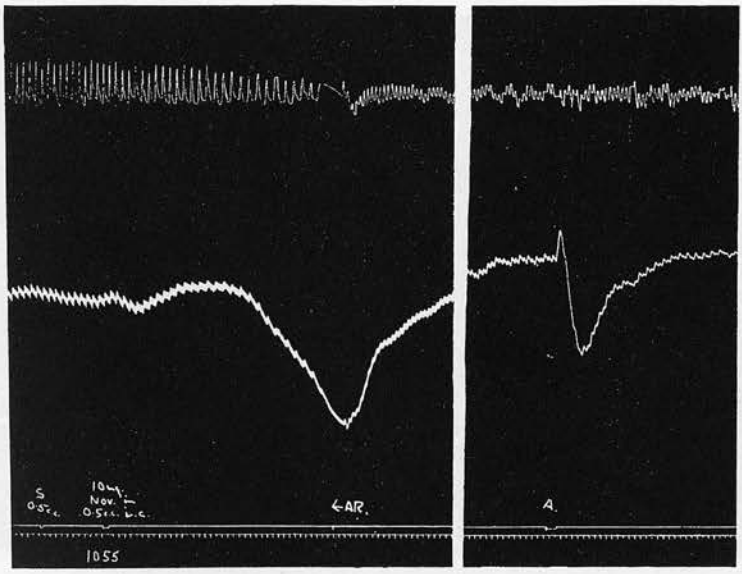

FIG. 2. ♂, 2500 grams, March 15, 1932. Chloralose. *A*, an intracisternal injection of 0.5 cc. saline is seen to be without apparent effect on blood pressure or respiration. When the injection contains 10 mgm. novocain HCl there is in this case only a slight rise of blood pressure and a steep and early fall with failing respiration. Positive ventilation shows a rapid recovery of the circulation. In this animal, as shown in *B*, a small intravenous injection of adrenalin (0.002 mgm. *A*) was vaso-dilator, and this presumably explains the difference between figs. 1 and 2, *A*.

tered by intra-cisternal or intravenous injection, is without apparent effect on the vasomotor center, as judged by a blood pressure record.

2. The action of novocain when injected in the lumbar region. Provided the volume of the injection be restricted, relatively large quantities of novocain can be injected into the cerebrospinal fluid bathing the lower parts of the cord without producing symp-

toms other than the abolition of spinal reflexes, (which is usually desired) and a fall of blood pressure to a level of 70 or 80 mm. Hg. In an experiment in which the spinal canal was opened and the cord transected at the level of the seventh cervical vertebra novocain when freely infiltrated by lumbar puncture (100 mgm.) did not reduce the blood pressure below 80 mm. (c. f. fig. 3). It

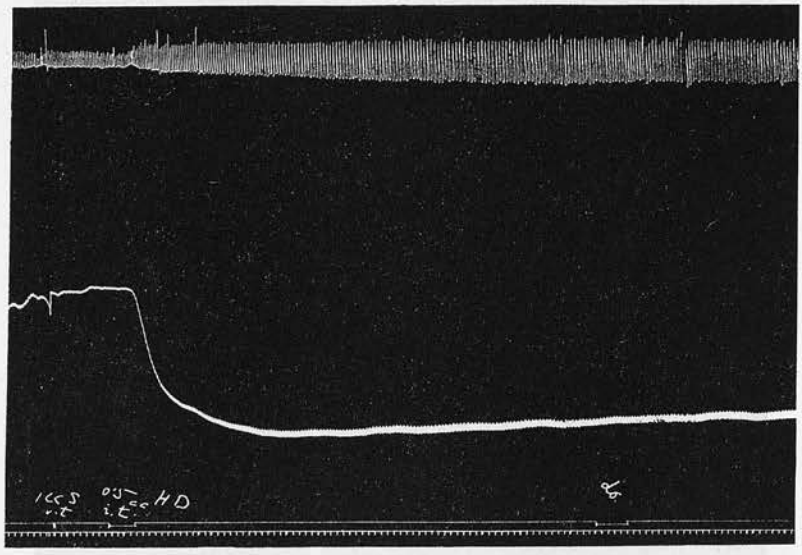

FIG. 3. ♀, 2100 grams, November 26, 1931. Dial. The spine of the first dorsal vertebra has been removed and the membranes opened to prevent spread of the intrathecal injections above this level. Intrathecal injections are shown of 1 cc. saline, and two injections of 0.5 cc. "heavy duracain," each containing, in a viscous solution, 50 mgm. novocain. The first injection of novocain produces a precipitous fall in blood pressure, and the diaphragmatic movements are increased with the paralysis of the thoracic movements. Repetition of the dose is without apparent effect. Three similar injections had been given about two hours earlier, and recovery from the effects on both occasions was apparently complete within ninety minutes.

is only when the drug, either because of repeated or voluminous injections, reaches the level of the phrenic roots or the medullary centers, that the life of the animal is endangered.

The paralysis, by novocain, of spinal vaso-constrictors, may contribute to respiratory failure by reducing the blood supply to the medulla, but the toxic effects must be exerted mainly on the

respiratory mechanisms, since adequate ventilation never fails to resuscitate the animal. In an animal supplied throughout with artificial respiration, very large amounts of anesthetic can be injected without serious effects, just as, intravenously, novocain can be infused to the extent of 12 or 15 times the amount required to paralyse respiration before the heart fails (Macdonald and Israels, 1932).

In surgical spinal anesthesia the depression of the circulation due to the paralysis of spinal vaso-constrictors is often enhanced by the exposure of and traction on the abdominal contents. We have made the same observation in the course of our experiments.

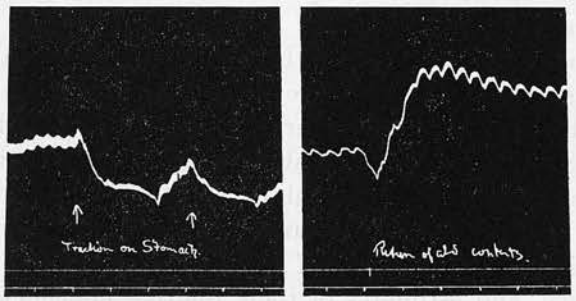

FIG. 4. ♂, 3050 grams, March 18, 1931. Chloralose. An intrathecal injection of 25 mgm. novocain HCl had reduced blood pressure from 140 to 85 mgm. Hg. Free exposure of the abdominal contents reduced the pressure to 70 mm. Hg, and traction on the stomach to 40 mm. Hg, as shown in *A*. (Traction at the arrows.) On the return of the abdominal contents to their cavity (*B*), the pressure promptly rises from 65 to 105 mm. Hg.

In one such case (fig. 4) the spinal anesthetic reduced the blood pressure to 80 mm. Hg. The abdomen was opened, the stomach manipulated, the duodenum mobilized and the gut freely exposed; each of these manoeuvres caused a further depression in the blood pressure, which fell on several occasions below 40 mm. Hg. The replacement of the abdominal contents was followed by a rise of pressure from 60 to 110 mm. Hg. In another case after the intravenous injection of a unit of pitressin and 20 cc. of warm saline the pressure rose from 30 to 70, and on replacing the viscera from 70 to 115 mm. Hg.

3. Special novocain solutions and other local anesthetics. Clini-

cally, it is customary to use, instead of simple solutions of novocain in saline, solutions containing a viscous substance such as starch or gliadin to "bind" the anesthetic solution and limit its diffusion. In the experimental animal we have satisfied ourselves that with such solutions much larger doses of the active drug can be given without respiration being affected. On the other hand, if paralysis does ensue, it takes much longer to disappear. Thus two hours or more of artificial respiration may be necessary. The use of strychnine in such solutions seems difficult to justify on theoretical grounds, and we have seen no advantage from its presence. Alcohol is often added to diminish the density of the solution, but the use of a heavy solution is becoming increasingly popular in the hospitals here.

Our experimental findings with novocain have been duplicated with stovain and percain. The anesthesias and paralyses produced by these drugs have appeared to be more profound and more lasting. Stovain is effective in about half the dose that is required with novocain, and percain in about one-twelfth of the novocain dosage. It is obviously difficult to establish, experimentally, the superiority or inferiority of one drug to another. All these are effective, and estimates of their relative efficiencies depend upon their relative toxicities and the incidence of undesirable sequelae, as observed after their use.

4. Preventive and restorative measures against novocain poisoning. It is common to use elaborate pre-medication in clinical spinal anesthesia. Apart from morphine to ease the patient and atropine to quieten abdominal movements and reduce secretion, ephedrine is often given to help to maintain blood pressure. We have not fully investigated its value experimentally, but we have satisfied ourselves that pre-medication with ephedrine, adrenaline, pituitrin and caffeine, even in heroic doses, cannot prevent respiratory paralysis following the intrathecal administration of novocain in paralysing amounts, nor can they save the animal when administered on the first signs of the appearance of such paralysis. If the artificial respiration has been delayed and the heart is failing, intravenous or intra-cardiac adrenaline may be most helpful. In several cases adrenaline and cardiac message through the chest

wall have revived a heart that has stopped, but it cannot be too strongly emphasized that artificial respiration, early and adequate, is the *sine qua non* in saving the animal, and if started in time it is an unfailing and sufficient cure in itself. Post-pituitary extract, because of its sustained and direct action on the blood vessels, especially when combined with intravenous saline, is useful, and like caffeine speeds the return of abolished reflexes, such as the knee-jerk and corneal reflex, and may accelerate the return of spontaneous respirations.

In one experiment in which a liberal dose of pitressin was used the paralysed respiratory center failed to recover after two and a half hours of artificial respiration, although the blood pressure was quite satisfactory. It may be that in this case the closure of the medullary vessels was such as to cause permanent injury.

Roeder (1931) lays much importance on the administration of 5 to 10 per cent of carbon dioxide in oxygen. He claims that the respiratory activity thus provoked was sufficient in 2 cases to maintain "enough circulation for vital purposes without any assistance from the myocardium." In our experiments no support for such a thesis has appeared, nor can vascular collapse be established apart from respiratory paralysis, and our view is that in the cat myocardial ischemia never precedes a more serious medullary ischemia. While not wishing to under-estimate the effects on the heart of variation in intra-thoracic pressure, we are unable to think of the movements of the thorax as functioning, in any real sense, as a circulatory pump.

There is, however, no doubt that the administration, by artificial respiration, of 5 per cent carbon dioxide in oxygen is much more efficient in accelerating recovery and maintaining a good circulation than is air, but it is no preventive of paralysis.

One cannot imagine that a respiratory stimulant is capable of preventing the action of a powerful depressant, administered in paralysing doses, any more than pilocarpine can antagonise the toxic actions of full doses of atropine.

In man the tilting of the operating table to put the patient in a 5 to 10° Trendelenberg or head-down position helps the venous return and reduces any tendency to fainting. In the cat the

relation to the problems of spinal anesthesia. It is shown that the incidence of a dangerously toxic action is always, in the long run, on the respiratory system, although circulatory depression may contribute. In the cat adequate and timely artificial respiration serves as a reliable antidote to novocain poisoning.

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THE ACTION OF LOCAL ANAESTHETICS ON THE RESPIRATORY APPARATUS

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INTRODUCTION

In a previous paper (1) we have indicated that in our experience in the experimental animal, the introduction of solutions of local anaesthetics into the cerebro-spinal fluid whether by lumbar or cisternal puncture, endangers the *life* of the animal in so far as it acts on the nervous control of respiration. Such control may be affected in several ways: through depression of the respiratory centre, of the phrenic roots, of the spinal roots of the nerves to the accessory muscles of respiration, or through some combination of these factors. The literature contains such discrepant views as to the occurrence and relative importance of these possibilities both in the experimental animal and in human spinal anaesthesia that it has seemed to us to be desirable to re-examine the problems involved.

RESPIRATORY PARALYSIS AFTER INJECTION INTO THE CEREBRO-SPINAL FLUID

If a local anaesthetic drug such as procaine be given intrathecally, in sufficient dose, whether introduced in the lumbar region and spreading "too high," or deliberately injected into the *cisterna magna*, it will paralyse respiration. Is such paralysis central, that is, does the drug act on the respiratory centre, or is it a root paralysis, attacking either the phrenics or the anterior roots of the dorsal nerves supplying the intercostal muscles? There is both clinical and experimental evidence against direct paralysis of the respiratory centre. Spinal anaesthesia is gen-

erally accepted to be a root anaesthesia, not due to any action on the spinal cord itself. If a drug does not penetrate the cord under the ordinary conditions of spinal anaesthesia, why should it penetrate the medulla and attack the respiratory centre? Further, operations on the head have been carried out quite successfully under extended spinal anaesthesia. Thus Koster and Wolf report 27 mastoid operations under spinal anaesthesia without a fatality. This means that there must be a concentration of procaine hydrochloride, the drug they use, which will paralyse sensation but not the vital centres or the diaphragm. "It is this property of selectivity dependent on inherent differences in nerve fibres that participates in the explanation of surgical anaesthesia of the entire body including the head without respiratory or cardiac paralysis (3)." The other participant in Koster's explanation is his experimental work in which he found that the direct application of pledgets of cotton wool soaked in 2.5 per cent solution of procaine hydrochloride to the medulla and upper cervical cord did not paralyse respiration (7). Henderson and Johnston, not satisfied with the precision of such pledget experiments, found that the injection of 1 cc. of a 5 per cent solution of the drug into the fourth ventricle of the dog did not paralyse respiration either. The results of cisternal injections were variable. Such "at times cause little or no effect, or a rise of blood-pressure and a fast heart partly due to paralysis of the vagus, and in other cases relatively sudden death from respiratory failure" (4). Such respiratory failure they attribute to paralysis of the diaphragm, not of the respiratory centre: "This appeared to occur in a number of our cases both with injection into the cisterna and into the seventh cervical-first thoracic interspace." As regards the circulatory changes they describe, another explanation of these has been advanced by Dixon (5) and supported by Hill and Macdonald (1).

Similarly in cats Harrison and Frank (6) found that intracisternal procaine hydrochloride, even in 5.5 per cent (i.e. isotonic) solution, produced anaesthesia (in the absence of any other anaesthetic) without respiratory paralysis. One per cent solutions could be applied to the medulla and cerebellum for over an

hour without paralysis, and even when the cord was "edematized" with such a solution, paralysis took fourteen minutes to develop.

If we sum up this evidence we see that Koster and Wolf's experience is against the idea that procaine acts on the centre but it is equally against its action on motor roots. Henderson and Johnston and Harrison and Frank are united against the action of procaine on the centre, but the two former produce evidence of occasional failure which they attribute to the action of the drug on the phrenic roots.

Our experiments have been made on cats, and differ from those of Harrison and Frank in that we have worked on animals under chloralose as a light basal anaesthetic, as described in our previous paper, and we have introduced our drugs into the theca with a minimum of dissection or exposure—never cutting into the spinal column or cranium except to expose the fourth ventricle. We believe it is important to avoid exposure trauma and haemorrhage in such work, and have evidence that such factors profoundly modify the responses to our injections. We propose to summarize the results obtained in a long series of experiments, arranging them in accordance with the location of the injections.

INTRACISTERNAL INJECTIONS

The respiratory changes following intracisternal injections are remarkably constant; they vary in degree but not in kind. Thus a large dose produces immediate respiratory paralysis; with a moderate dose (5 to 10 mgm.) of procaine the paralysis develops in a minute or two; with still less (1 to 5 mgm.) respiration may be seriously depressed or its rhythm merely slowed.

LUMBAR INJECTIONS

When the drug is injected in the lumbar region, it will have no effect on respiration if it remains there but if it diffuses up the spine it paralyses the intercostals and the cat breathes with its diaphragm only; if the drug goes as high as the phrenic roots then artificial respiration may be required to save the animal's life. In this case even if the drug spread later to the medulla there would be no possibility of demonstrating respiratory failure since such already exists.

The changes in the respiratory records following such injections depend upon whether thoracic or abdominal movements are principally recorded. As in figures 1 and 2 we have often observed both. Intercostal paralysis naturally involves a marked reduction in thoracic excursions. Usually abdominal movements are increased in compensation—notably in figure 3 of our earlier paper (1). Occasionally such compensation involves a brief phase of acceleration as well as the usual increase in amplitude, presumably because of central stimulation following the thoracic paralysis, but such changes in the respiratory rhythm following lumbar injections are rare.

CERVICAL INJECTIONS

If 10 mgm. of procaine, preferably in a viscous solution, such as "Heavy Durocain," be injected into the theca just behind the body of the third cervical vertebra, the picture presented is almost the reverse of that seen after an ascending lumbar injection; the diaphragm is paralysed but the intercostals can compensate for its loss by increased activity. In an anaesthetized cat purely thoracic respiration is quite sufficient to enable it to survive until such time as the diaphragmatic paralysis passes off (fig. 1).

These experiments demonstrate that a healthy cat at rest can breathe efficiently either with its diaphragm or its intercostals. In such a cat an injection of procaine under the third cervical vertebra may paralyse the diaphragm, but the intercostals alone are able to sustain the life of the animal. If an hour or so later when the diaphragm has completely recovered its power, an exactly similar injection be made into the *cisterna magna*, both intercostal and diaphragmatic respiration are paralysed in anything from one to four minutes, and the animal dies, if not immediately rescued by artificial respiration (fig. 2b).

It seems impossible to avoid the conclusion that this is due to paralysis of the centre. It cannot be merely an intercostal paralysis because the injection is nowhere near the intercostal roots. It cannot be a phrenic paralysis because we have already proved that the cat's intercostals can sustain life. It could not be both at once because apart from the fact that it is nowhere

near either, it is far too quick (*cf.* figs. 1 and 2a with fig. 2b). It must therefore be a paralysis of the centre.

There is further evidence to the same effect. If the tracing of the respiratory failure which results from an intra-cisternal injection be compared with one that results from an injection under the third cervical vertebra, characteristic differences will be noticed. If the phrenics are paralysed the respiratory excursions traced by the recording tambour become progressively less

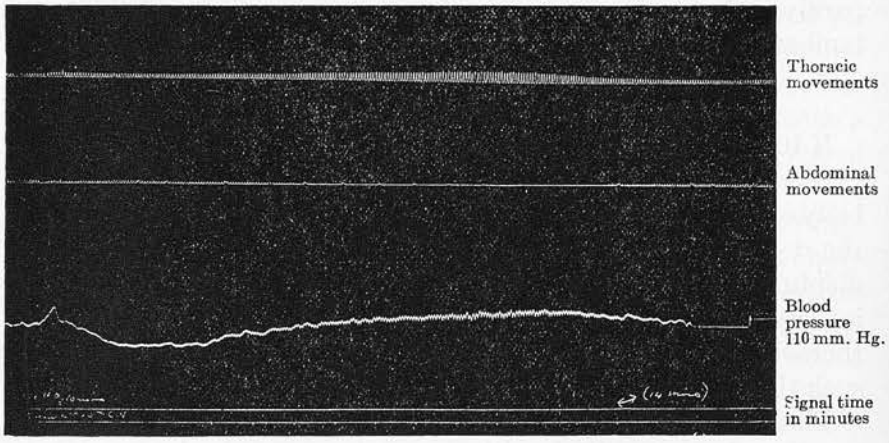

FIG. 1. CAT, ♀, 2650 GRAMS, CHLORALOSE

At signal, 10 mgm. procaine benzoate in 0.1 cc. were injected between cervical vertebrae 2 and 3. Diaphragm contractions are reduced and cease in eight minutes, during which period the thoracic movements increase to compensate for the loss of the diaphragm. Fourteen minutes after the injection the diaphragm begins to recover, and the amplitude of the chest movements rapidly returns to normal. The blood pressure changes are slight and transitory.

and less and cease in from five to seven minutes (fig. 1). The rate of respiration is not usually affected. If on the other hand it be the centre which is paralysed the depth of respiration is less affected but the *rate* is slowed (fig. 2b, see also fig. 1 of our earlier paper (1)). No such paralysis has taken more than four minutes to threaten a fatal result and generally much less. These facts serve to distinguish a central from a diaphragmatic paralysis and are clearly demonstrated in the accompanying figures.

In the light of the recorded experiments of others some diffi-

culty might be expected in paralysing the phrenic roots and leaving the intercostals working perfectly, but we have succeeded in six animals. In a heavily anaesthetized animal adequate compensation by the accessory respiratory muscles does not take place. The volume of spinal anaesthetic solution used should be

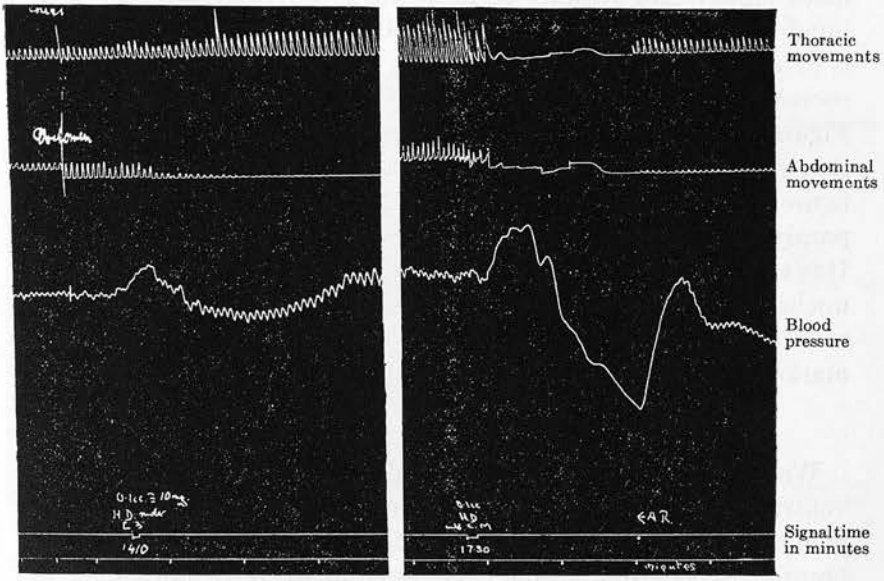

FIG. 2. CAT, ♂, 2100 GRAMS, CHLORALOSE

Comparison of the application of 10 mgm. procaine benzoate in 0.1 cc. by injection into the cerebro-spinal fluid; (a) under the body of the third cervical vertebra, (b) into the cisterna magna.

In (a) the diaphragm is rapidly paralyzed (two minutes) and the thoracic movements are increased in compensation. The blood pressure, as in figure 1, is little affected. In (b) all respiratory movements are first slowed and then paralyzed together, and the animal would die were it not supplied with artificial respiration (A. R.).

The two experiments are on the same animal with an interval of three hours between them.

small, and if a viscous solution be used diffusion will be less. For most of our experiments we have used 0.1 cc. of "Heavy Duracain"—a 10 per cent viscous solution of procaine benzoate. But we have also succeeded with a similar injection of the hydrochloride in simple aqueous solution. In some animals half the above dose is adequate. The cervical injection is given by clearing the

side of the spine of the axis, and directing a fine hypodermic needle between this and the third cervical vertebra, downwards and forwards, till the resistance of the body of the third cervical vertebra be felt. Unless cerebro-spinal fluid be tapped by the puncture it may be assumed to have failed. The injection is made slowly and steadily from an insulin syringe graduated in hundredths cubic centimeter. The subsequent cisternal injection is given as previously described (1), and tracings of two of the successful experiments are reproduced in text-figure 1 and 2a. Figure 2b shows for comparison the complete paralysis of respiration when a similar injection is introduced into the cisterna. In figure 2a the action is practically limited to a diaphragmatic paralysis with compensatory increase of the thoracic movements. It is to be noted that the rhythm of the respiratory movements is unchanged, whilst in figure 2b the complete paralysis of the respiration is preceded by some slowing. This slowing has been more marked in other tracings, notably in figure 1 of our earlier paper (1).

INTRAVENTRICULAR INJECTIONS

We have also introduced the local anaesthetic into the fourth ventricle directly by trephining the occiput and raising the lower pole of the cerebellum to allow of the entry of the point of a fine blunted hypodermic needle. Here again we have not failed to get prompt depression or paralysis of the central type (fig. 3). Owing to the exposure and haemorrhage the circulatory changes are more severe, but complete recovery ensues often in a remarkably short time. There can be no possibility of the spread of the drug to spinal roots with such intraventricular injection.

Since these observations are opposed to those of the authorities we have quoted, we have repeated the experiment on three dogs. In the dog instead of trephining we have found it easier to clear the muscles from the base of the occiput and remove the bone with nibbling forceps. The exposure of the ventricle is complicated by the extent of the venous plexuses, but with care serious haemorrhage can be avoided.

In a 5 kgm. dog, anaesthetized with morphia and chloralose, 0.4 cc. of 5 per cent procaine solution produced a marked slowing

of respiration and 0.5 cc. a prompt paralysis, as in the cat. On trying to repeat this observation in the same animal an hour and a half later, a similar injection was found to have no effect. The floor of the ventricle and presumably the centre were found to be protected by a coagulum—partly blood but mainly cerebro-spinal fluid—which was apparently not penetrated by the drug. On removing this film by gentle sponging and aspiration respiratory

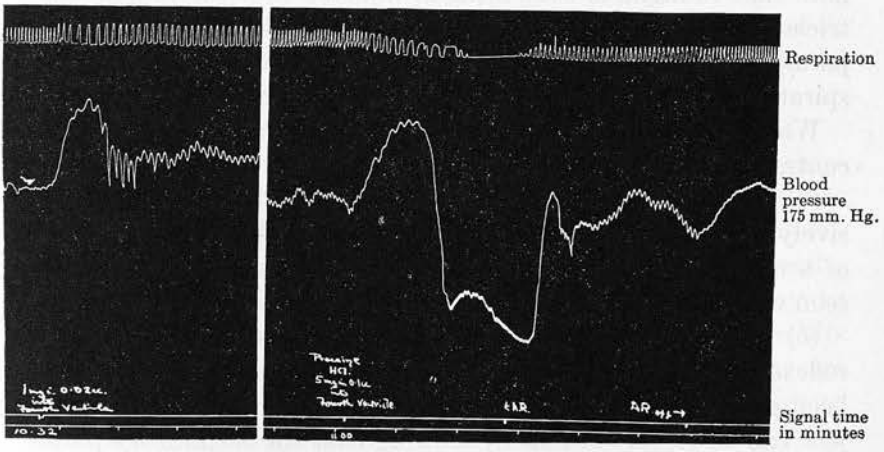

FIG. 3. CAT, ♂, 2800 GRAMS, CHLORALOSE

(a) 1 mgm. procaine HCl in 0.02 cc. placed in fourth ventricle. Respiration is slowed, its depth little affected.
(b) 5 mgm. procaine HCl in 0.1 cc. similarly applied. The slowing of the respiration passes into complete failure in two minutes, and death would ensue were artificial respiration not supplied. With three minutes of such ventilation, natural respiration re-appears, and the recovery as judged by the tracing is complete.

depression was again produced by a fresh application of local anaesthetic.

RESPIRATORY PARALYSIS WITH INTRAVENOUS INFUSION

When a solution of a local anaesthetic is infused intravenously into an experimental animal, as in the method for the estimation of the relative toxicities of such drugs, as recommended by Macdonald and Israëls (2), a point is reached at which respiration fails, and unless artificial respiration be instituted the animal perishes.

The dose required to bring about the fatal result is in the neighbourhood of 50 to 70 mgm. per kilogram. The actual figures in one animal were as follows: (*a*) 68.1 mgm. per kilogram at the rate of 2.62 mgm. per kilogram per minute. (*b*) 62.4 mgm. per kilogram at the rate of 2.12 mgm. per kilogram. per minute. (*c*) 73.5 mgm. per kilogram at the rate of 2.73 mgm. per kilogram per minute. In striking contrast to these enormous doses, we note that 10 mgm. or even 5 mgm. injected into the fourth ventricle or intracisternally will kill a cat in about two minutes by paralysis of respiration. How then does the infusion paralyse respiration? Does it act on the respiratory centre or peripherally?

We believe the paralysis caused by the intravenous infusion is central for the following reasons:

(*a*) As the infusion progresses the respiration becomes progressively *slower rather than shallower*, a state of affairs characteristic of a central as distinct from phrenic paralysis, as we have just seen when the drug was applied to the centre.

(*b*) Even when respiration has been paralysed, various spinal reflexes can be elicited. Thus in a cat in which respiration has been paralysed by such an infusion, the knee jerk and the elbow jerk can be readily obtained, showing that the respiratory paralysis is unlikely to be due to the effect of the drug on any point in the ordinary spinal reflex arc.

(*c*) The duration of the induced paralysis is brief. Usually if respiration be just abolished one minute of artificial respiration is enough for the return of the spontaneous movements of the muscles of respiration. In one of the experiments referred to under (*b*) above, in which the infusion was continued till twice as much had been given as was required to paralyse respiration, with artificial respiration during the second half of the infusion, recovery was sufficiently developed for natural respiration to appear normal within five minutes. Peripheral paralysis, in our experience, if developed under a local anaesthetic, lasts longer.

DISCUSSION

It is not easy to find a satisfactory explanation for the discrepant findings as to the effects of local anaesthetics when these

are introduced into the upper parts of the spinal canal. Our own animal experiments are open to the criticism that we have always added the effects of such injection to the depression already present due to the chloralose or whatever other basal anaesthetic we have used. But hypnotic premedication before spinal anaesthesia is almost a routine practice in man. If morphine for example has been administered before the spinal puncture and if the subsequent local anaesthetic spreads high its action on the respiratory apparatus will be additive to that of the morphine on the centre. On the other hand in unanaesthetized animals the puncture of the spinal canal must often be difficult, not to say uncertain, as regards the fixation of the precise location of the point of the needle. If this point does not lie free in the cerebrospinal fluid—if the fluid does not leak through the needle—if the fluid be blood-stained—our experience is that all such experiments are likely to be unsatisfactory. The varying results obtained by certain workers, as in (4) quoted above, may be explicable on some such basis. In our experiments the results are reasonably uniform and predictable. In particular we are at a loss to understand the failure of the local anaesthetics to affect the respiratory centre when introduced directly into the fourth ventricle, unless it be that the injection has failed to reach the ventricle, or that, although the drug be there, the centre lies protected by a film of blood clot or other coagulum. Johnston and Henderson tell us "High concentrations in the fourth ventricle do not produce death" (4). So far as the cat and dog are concerned we can contradict this statement, and we regard it as untenable on both theoretical and experimental grounds, in the light of evidence which we have advanced.

In human spinal anaesthesia, when the drug is introduced by lumbar puncture, the main risk lies clearly in a progressive ascending paralysis of the spinal roots of the respiratory muscles. This has recently been emphasized by Sebrechts (8). Since this in itself will be fatal unless adequate artificial respiration be promptly supplied, a later possible paralysis of the centre need hardly be considered. Except where some gross error in dosage or technique has been committed, or where some hypersuscepti-

bility exists, the risk of central paralysis in man is remote, and we doubt whether satisfactory evidence of such occurrence has yet been advanced. We would regard it, however, as at least a theoretical danger in cases of "complete" spinal anaesthesia as practised by Koster and his associates.

SUMMARY

The mechanism by which the local anaesthetics may produce respiratory failure varies with the route by which they are exhibited. Evidence is given for a depression of the respiratory centre as the main action when the drug is absorbed into the circulation or introduced into the *cisterna magna* or fourth ventricle. When the drug is introduced by lumbar puncture the greater danger lies in an extension of its action to the nerve roots of the respiratory muscles, and especially to the phrenic roots.

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THE NEUROLOGICAL SEQUELÆ OF SPINAL ANÆSTHESIA

By Dr. A. D. MACDONALD

Experimental and Pharmacological Considerations.—I am neither a neurologist nor an anæsthetist, but have the good fortune, for a pharmacologist, to have a number of clinical colleagues who come to my laboratory to discuss their doubts and difficulties and subject their theories to experimental investigation. While I would be the last to suggest that we should blindly apply the findings on cats and dogs to man, I think that when faced with such problems as have been outlined by the opener of this discussion, we must explore every possibility of precise knowledge.

It would be unprofitable to attempt to review the published experimental work on spinal anæsthesia. Much of it is overwhelmingly conflicting, much is certainly erroneous in its conclusions. Instead I wish to summarize three pieces of joint research work which have been carried out in my laboratory—the first two inspired by a question of Dr. Falkner Hill's—" Why do spinal anæsthetics sometimes cause death on the table "—the last, which bears very directly on to-night's discussion, raised by Mr. Kenneth Watkins, " Why do we find bladder, rectum, and sensory disturbances persisting after spinal anæsthesia ? " Two of these researches have not yet been published.

A glance at a skeleton of a cat or dog will show that lumbar puncture above or below the last lumbar vertebra should be easy, and with a little practice it is not difficult to tap clear cerebrospinal fluid. Because of the continuations from the cord into the tail, there is much less space than in man, but in our experiments we reject any in which a free flow of cerebrospinal fluid is not obtained, and any in which the fluid continued to be tinged with blood.

In the first place Dr. Bullock and I have tried to determine the fate of the injected drug—its spread in the cerebrospinal fluid, its dilution, absorption, destruction, and excretion. So far we have limited our work to drugs of the para-amino-benzoic acid series—procaine, larocaine, and tutocaine. These have the advantage of a reactive amino-group, and when coupled with guaiacol disulphonic acid yield an orange-coloured compound the concentration of which in cerebrospinal fluid, blood, urine, and tissues can be estimated colorimetrically to within 5%, although only small amounts are present. It is clearly important to make certain that the method is estimating the anæsthetic and not some derivative such as para-amino-benzoic acid itself. We have satisfied ourselves as regards concentrations persisting in the cerebrospinal fluid, by checking the colorimetric estimations with an assay against procaine, using the duration of anæsthesia produced by intracutaneous injections as a measure. A very good agreement was obtained.

Most anæsthetists who use procaine for spinal anæsthesia inject up to 3 c.c. of a 10% solution. Some dilute the drug further with cerebrospinal fluid before injection. In the cat 0·5 c.c. of a 10% solution may be injected without endangering respiration. This is about five times the full human dose, calculated on a basis of body-weight, yet it rarely affects the fore-limb tendon reflexes, though giving complete paralysis below that level for thirty to fifty minutes.

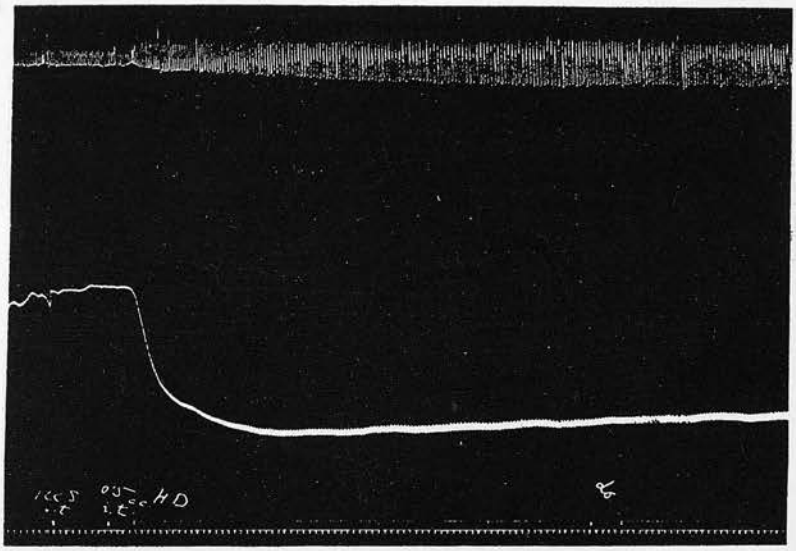
If small samples of cerebrospinal fluid are collected from the lumbar region at intervals and the drug content estimated, it is found that the concentration falls rapidly at first, then gradually. After five minutes it is usually between 2 and 3%. If a sample be taken at this stage from the cisterna magna, the concentration of drug there is surprisingly low—the highest we have found is 0·002%. One would not expect 6th-nerve palsies with such concentrations. After forty minutes or so, when the knee-jerk is back, the concentration in the lumbar region is usually below 0·05%. Larocaine and tutocaine are more potent and more toxic drugs, and give a longer anæsthesia after the injection of smaller doses. With larocaine the knee-jerk returns when the lumbar concentration has fallen to between 0·007 and 0·015%. With tutocaine the corresponding figures were 0·004 to 0·01%. (Slides showing the curves for the rate of disappearance of procaine, larocaine, and tutocaine from the lumbar cerebrospinal fluid.)

The concentration which is found in the blood at any time during or after spinal anæsthesia is low. It should not be necessary to-day to discount the fable that spinal anæsthesia may be dangerous because of the risk of blood absorption ; both clinical and experimental evidence disprove it. Thus Hill has injected a full spinal anæsthetic dose of procaine hydrochloride, 300 mgm., intravenously in man, spreading the injection over a few minutes, without producing detectable symptoms, and Sebrechts has reported a similar experiment with percaine. In the cat the liver may contain about twice as much as the blood ; the concentration in the urine has never exceeded 0·03%, and only about 7% of the total injected is excreted by the kidneys—the rest is presumably broken down by the liver.

In our work on the acute effects of spinal anæsthesia (Hill and Macdonald [1933 ; 1935]) we gave repeated lumbar injections of 0·5 c.c. of a 10% solution of procaine. We had taken steps to prevent the drug from reaching the phrenic roots or respiratory centre by removing the spine of the 7th cervical vertebra and passing a drain round the cord. Only the first dose produced demonstrable effects. The respiratory tracing (fig. 1) is taken from the diaphragm, and with the injection the excursions recorded by the tambour are increased. This is because the intercostal muscles have been cut off by the spinal, the diaphragmatic movements increasing to compensate for the loss of thoracic movements. The blood-pressure falls abruptly with the spinal, but only to 70 mm. Hg. Later injections do not lower the blood-pressure further. We have found, in fact, that in the lightly anæsthetized cat this rather terrifying fall of blood-pressure does not occur if the injection be made slowly, so that there is time for compensating reflexes to make the necessary adjustments for the paralysis of the lower vasomotor roots. In most clinical cases, in which procaine is used, the anæsthetic in itself need not depress the blood-pressure.

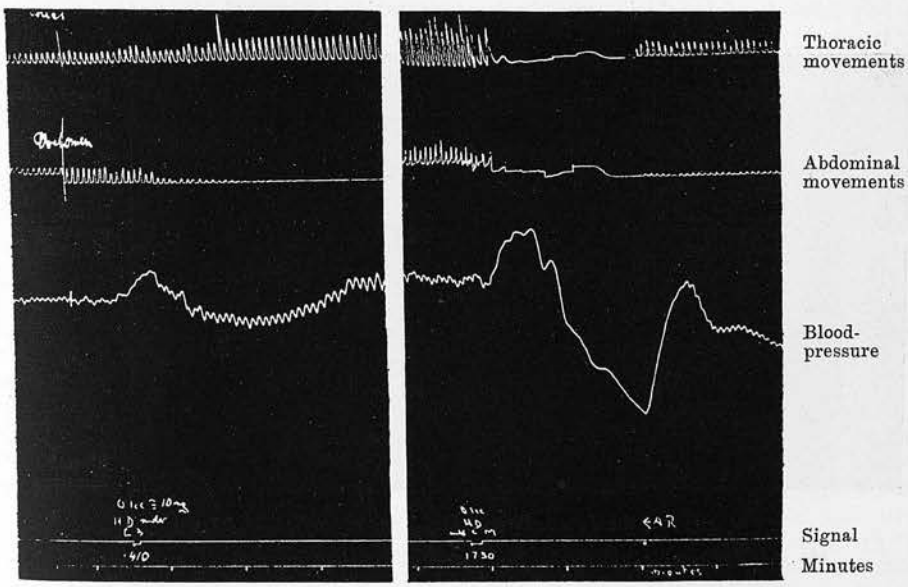
Here two respiratory tracings (fig. 2) are shown—the upper thoracic (intercostal), the lower abdominal (diaphragmatic). The injection (0·1 c.c. of 10% procaine) is first made at the level of the phrenic roots—between the third and fourth cervical vertebra. In contrast with the previous record, phrenic paralysis is produced, with compensatory increase in intercostal movements. Blood-pressure is little affected. For comparison, I have injected a similar dose into the cisterna magna of the same animal three hours later. This produces complete respiratory paralysis, the blood-pressure begins to fall, and the animal would perish were it not rescued by a few minutes of artificial respiration. In less than five minutes the depressed centre has recovered sufficiently from the effects of the drug to function independently.

Figure 3 is a more instructive tracing of a cisternal injection with respiratory paralysis, because the paralysis develops more gradually. There is a considerable rise of blood-pressure at first—a non-specific effect described by Dixon and attributed to stimulation of a suprarenal centre. The respiratory paralysis here is strikingly different from the phrenic paralysis seen in the last slide. Here the rhythm rather than the amplitude of the excursions is affected—the paralysis is central, not



[From the *Journ. Pharm. and Exp. Therap.*, 1933, **47**, 156

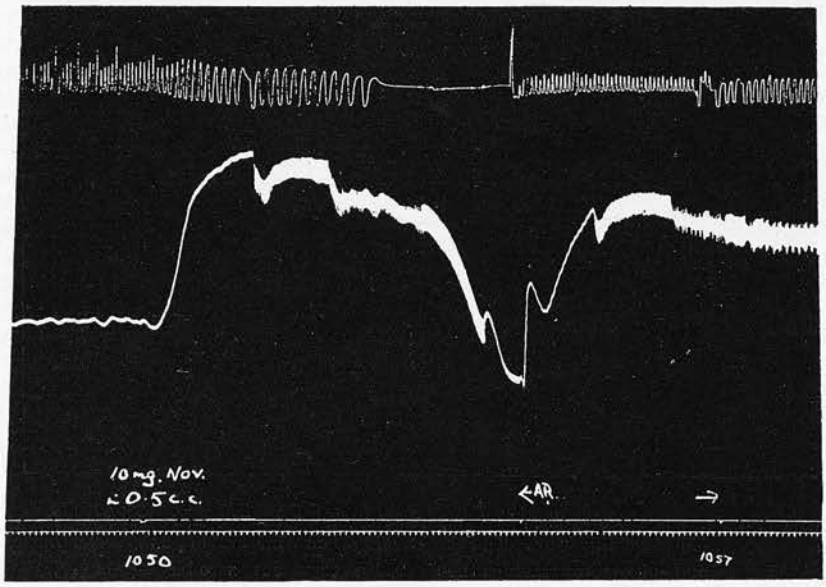
FIG. 1.—Lumbar injection. The time-tracings show intervals of five seconds. (In this and subsequent tracings the signal line represents zero blood-pressure.)



[From the *Journ. Pharm. and Exp. Therap.*, 1935, **53**, 459.

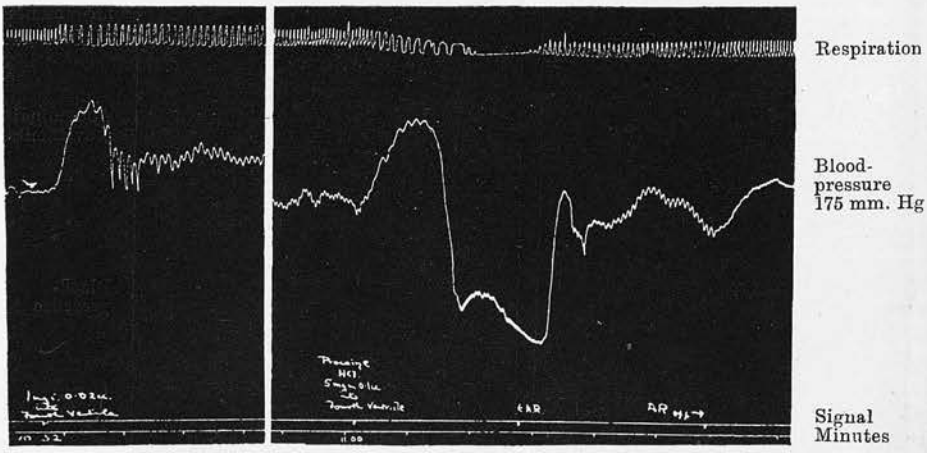
FIG. 2.—Cervical injection, and for comparison cisternal injection. The time-tracings show intervals of one minute.

peripheral, and every discharge from the centre, until it is paralysed, produces a full, deep respiration. Here again a very short spell of artificial respiration serves for the centre to recover its normal rhythmical activity.



[From the *Journ. Pharm. and Exp. Therap.*, 1933, **47**, 154.

FIG. 3.—Cisternal injection. The time-tracings show intervals of five seconds.



[From the *Journ. Pharm. and Exp. Therap.*, 1935, **53**, 461.

FIG. 4.—Ventricular injections. The time-tracings show intervals of one minute.

Here two injections into the fourth ventricle are recorded (fig. 4). After trephining and improving the access, the lower pole of the cerebellum is raised and a blunt hypodermic needle is passed up to the ventricle. With 1 mgm. of procaine, respiration

is slowed but not paralysed. With 5 mgm. a typical central paralysis, similar to those described with cisternal injections, is produced. Recovery with artificial respiration is rapid. The vasomotor disturbances associated with these central paralyses are transitory and not alarming—there is no evidence of any depression of the vasomotor centre similar to that of the respiratory centre.

So far, then, as we can judge from the experimental animal, the acute danger of spinal anæsthesia consists of the risk of headwards spread of the anæsthetic from the point of injection, depressing in turn the thoracic muscles, the diaphragm, and finally the respiratory centre. So long as it is possible to initiate and maintain adequate and timely artificial respiration, such paralysis does not seriously threaten the life of the animal. In avoiding respiratory paralysis, the important factor seems to be to limit the bulk of the injection—a voluminous dilute injection is much more likely to reach the phrenic roots or even the respiratory centre than an equal weight of drug in a more concentrated solution. But we shall see that a concentrated solution is not without its drawbacks.

When Watkins and I began our study of the neurological sequelæ in the experimental animal, we realized that to obtain symptoms in a reasonable proportion of our animals heavy doses would have to be given. We began with ½ c.c. doses of various 10% solutions of procaine—about five times the maximal clinical dose. In a fair proportion of animals this produces lasting symptoms—sensory disturbances, drooping of part of the tail, or complete flaccidity, paralysis of the urinary musculature with distension, so that urine could be expressed by abdominal pressure, the stream stopping as soon as the pressure was discontinued, rectal weakness with some protrusion of the mucosa through the anus, and occasionally some weakness of the hind limbs. Here we were concerned with the question—were these changes due entirely to the local anæsthetic, or to the presence of some other constituent of the solution such as alcohol or glycerine? A further possibility consists of some potentiation of the anæsthetic by such constituents. We satisfied ourselves that neither the alcohol nor the glycerine, in strengths greater than are commonly employed in proprietary solutions, produce sequelæ in themselves. Further, since simple solutions of procaine may provoke lasting symptoms, it seems unnecessary to postulate some obscure potentiation.

Table I summarizes our results for several brands of procaine in 10% solution.

TABLE I.—SUMMARY OF EXPERIMENTAL RESULTS.

	Total	Normal	Paralysis	Percentage paralysis	Full	Tail only
Alcohol and glycerine (15 to 20% each)	23	23	—	—	—	—
"Heavy" duracaine (10% planocaine)	23	10	13	56	9	4
10% procaine (planocaine brand) ...	10	5	5	50	2	3
10% procaine (novocain brand) ...	23	17	6	26	4	2
5% stovaine (Barker)	10	4	6	60	—	6

We do not suggest that any significance should be attached to differences in the incidence of sequelæ with the various procaine solutions, and it is interesting to find that 5% amylocaine (stovaine) seems to be as likely to leave sequelæ as the stronger procaine solution.

After discussing these results with Dr. F. R. Ferguson we decided that we should try to correlate the incidence of sequelæ with the concentration of the drug we had injected. That a correlation exists is clear from Table II. With 2½%, no symptoms

TABLE II.—RELATION OF DOSAGE TO INCIDENCE OF SYMPTOMS.
(*Procaine hydrochloride*)

Concentration (%)	Number of animals	Number showing some paralysis	Percentage paralysis
2·5	20	0	0
5	20	2	10
10	56	24	43
20	8 (3 died acutely)	4	(80)

appeared. With 5%, 2 of 20 cats had residual tail droop. The 10% injections are collected from Table I. I then tried a small series with 20%. The first two given 0·5 c.c. died in a few minutes, presumably from respiratory paralysis. I therefore reduced the dose to 0·3 c.c. or 0·4 c.c. according to body-weight, but lost another animal acutely. Of the five surviving, four showed considerable paralysis—weakness of the tail and hind limbs, some protrusion at the anus, and vesical dilatation. In this series simple procaine solutions were used, and the absence of prolonged changes with the weaker injections, sharply contrasted with their frequency with concentrated drug, is striking. This series provides further evidence that it is the local anæsthetic and not the other constituents of the solution or mechanical trauma due to the needle which provoke trouble. It would indeed be difficult to connect a prick of the cord or roots with the extensive symptoms sometimes seen.

We had at one time hoped to investigate the intrathecal morbid histology of the affected animals, but while realizing the importance and value of this line of work we are not at present prepared to discuss it.

In conclusion, if I may say a word about the experimentalist's outlook on the problems which Dr. Critchley has outlined, it seems to me that there are two pieces of well-established work that we should bear in mind. The first is Sherrington's proof that changes in the nervous system are more easily produced at the nerve-cells and synapse than in the actual nerve-fibre. The second is Gasser's proof that, of nerve-fibres, the smallest in cross-section are the most susceptible to the action of cocaine and its substitutes. These conclusions may help in evolving a satisfactory explanation of the phenomena under discussion.

[For permission to reproduce the tracings (figs. 1 to 4) the editors are indebted to the *Journal of Pharmacology and Experimental Therapeutics*.]

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THE FATE OF DRUGS USED IN SPINAL ANAESTHESIA

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INTRODUCTION

The value of spinal anaesthesia as a method in man is now generally admitted, although, as with other methods, it presents its own difficulties and dangers as well as its advantages. The routine for its induction, the choice of local anaesthetic drug, the preparation of the solution, the technique of administration—these are by no means standardised. While it is clear from the differences in routine adopted by successful anaesthetists that it would be unwise for anyone to attempt to lay down what Babcock (1934) calls "the magical gesture or hocus pocus of safety and efficiency" it will be equally clear that some experimental method should be developed by which reasonably reliable comparisons might be made, at any rate of the drugs used. In the present paper some such comparison has been attempted for certain drugs of the para-amino-benzoic acid series—procaine (ethocaine, novocaine, etc.), larocaine and tutocaine. Observations have been made on the duration of the anaesthesia produced, the spread of the drug within the cerebro-spinal fluid (C.S.F.) and its dilution there; its absorption into the blood-stream, destruction in the tissues and excretion in the urine in a series of cats. As much of such work is involved in any consideration of the pharmacology of the local anaesthetics no matter by what route they have been exhibited, special attention has been paid to the changes in concentration of the drug in the cerebro-spinal fluid (C.S.F.) in the neighbourhood of the injection.

In a study of the changes in concentration of the drug in the C.S.F. of a small animal, such as a cat, in which it is not desirable

to withdraw for analysis more than 0.2 cc. of C.S.F. for any one estimation it is evident that a reliable micro-method is required. The usual assay processes of shaking out with solvents and weighing the drug or titrating with bromine are out of the question. Nephelometric and colorimetric methods had to be considered.

LITERATURE

Achard (1919) estimated the excretion of procaine in urine by the degree of turbidity produced with Tanret's reagent—a rough comparison using a reagent which is a general precipitant for alkaloids and proteins and in no sense specific for procaine—it had been used for stovaine by Desplas et Millet (1918). The "rhythm of elimination" for the two drugs was the same—most was found in the sample collected between the first and second hours after administration. Willstaedt (1934) describes precipitation reactions with various metallic ions, anthraquinone β-sulphonic acid, Reinecke salt, etc. Our own attempts at nephelometry with stovaine were so discouraging that we abandoned the method.

Most of the published colorimetric methods depend upon the free amino-group in the molecule of this series. Such a molecule is readily diazotized and when coupled with a phenol gives a strongly coloured dye and the reaction being quantitative, estimations can be made on low concentrations. The variations in the recommended methods depend upon the phenol which is used.

β-naphthol is employed in an identity test for procaine by the British Pharmacopoeia (1932). This reaction had been adapted to the quantitative estimation of procaine in C.S.F. by Küstner and Eissner (1930), who claimed that the method was sensitive to 0.1 mgm. and that no interfering substances were present in C.S.F. They concluded that the greater part of an injection of procaine left the C.S.F. within three minutes. Labat (1928) had maintained that "Chemical tests for novocaine in the lumbar region remain positive during the entire period of anaesthesia, while specimens of C.S.F. taken in the dorsal region, higher than the level of the anaesthesia, are free from the drug or contain

only very weak traces of it." Labat gives no actual figures, but his findings as regards absence of spread have been supported by Thompson (1934).

The main difficulty in the use of β-naphthol as the phenolic reagent is that the resultant dye is relatively insoluble and may separate out if the solution be at all concentrated. This can be overcome by the introduction of sulphonic groupings into the molecule as in aluminol and guaiacol 3-sulphonic acid. Aluminol —the sodium salt of β-naphthol disulphonic acid—was used by Kircher (1928) in detecting procaine in cocaine, but he does not mention any quantitative application of the reagent to the estimation of procaine. The same reaction was used by Popp (1928) in his investigations of cocaine and procaine in post-mortem specimens. Guaiacol 3-sulphonic acid was used as phenolic reagent by Cheramy in investigating the decomposition of procaine on heat sterilisation on the grounds that the colour developed much more slowly in the decomposition products than in the anaesthetic itself.

While our experiments were in progress Koster, Shapiro and Posen (1936) published a micro-method for the determination of procaine in C.S.F. based on the interaction with vanillin in dilute sulphuric acid to produce a yellow compound, as described by Maesemacher and Lucas (1931). In this reaction the presence of para-amino-benzoic acid need not vitiate the result. Riegel (1926) treated an acid solution of procaine with sodium nitrite and Nesslerized, matching the yellow colour produced on adding ammonia against standard bichromates.

METHODS AND FINDINGS

The technique of spinal puncture and anaesthesia in the cat has been described by Hill and Macdonald (1933, 1935). Samples of C.S.F. can be collected from the fine needles used for puncture into 0.1 or 0.2 cc. blood pipettes. The collection is very easy from the cisterna magna, rather slower from the lumbar or dorsal regions, but usually 0.2 cc. can be collected in two minutes. The first drops emerging are not collected as they might not be a fair sample. Indeed Bungenberg de Jong (1930) has criticized

Küstner and Eissner (1930) for such sampling, but they proved that any error so introduced is negligible. When samples of urine are required a urethral catheter is passed. Blood samples have been collected from the femoral artery—usually at the end of the experiment—and liver when required for analysis has also been collected at this time.

For the estimation of the local anaesthetic present in the body fluids or tissues preliminary experiments were carried out using β-naphthol:6:8 disulphonic acid as phenolic reagent for diazotisation. Although this yields a strong red colour proportional to the concentration of drug present, there are two objections to its use. Firstly a blank has a greenish shade so that the estimation had to be done with a tintometer rather than a colorimeter. Secondly even the purest chemical obtainable was fluorescent and this increased the difficulty of the estimation. This reagent was, therefore, given up in favour of guaiacol 3-sulphonic acid which gave only a trace of colour in the blank and a clear nonfluorescent dye. The colour is less marked than with the other reagent but will easily indicate a concentration of procaine in the C.S.F. under 0.005 per cent. Tutocaine and larocaine, like procaine, have a free NH_2 group attached to the benzene ring, and can be estimated by the same technique.

The routine of estimation for samples such as C.S.F. which are free from interfering substances was as follows:

Reagents: 1. HCl solution: 9 per cent concentrated HCl.

2. $NaNO_2$ solution:1 per cent $NaNO_2$.

3. Phenolic reagent: Potassium guaiacol sulphonate 1 per cent in Na_2CO_3 5 per cent.

4. Standard colour: $K_2Cr_2O_7$, 4 per cent.

Process: To 2 cc. of the test solution, usually made up from 0.1 or 0.2 cc. C.S.F. add 0.2 cc. HCl solution and 0.2 cc. $NaNO_2$ solution. Mix and allow to stand for 2 minutes; then add 0.2 cc. of phenolic reagent and repeat this latter addition in 15 seconds. Shake and at the end of 5 minutes more, match against the standard in a suitable colorimeter. The instrument used in this work was the Hellige wedge colorimeter, fully described by Autenrieth (1928). A preliminary series of estimations was carried

out using known concentrations of procaine and a graph constructed relating the scale reading with the procaine concentration. Similar graphs were prepared for tutocaine and larocaine. The accuracy of the method was tested by submitting twelve tubes of the same dilute procaine solution to the procedure. The results in milligram procaine per cubic centimeter were: 0.036, 0.038, 0.0365, 0.0377, 0.036, 0.0361, 0.0385, 0.036, 0.036, 0.036, 0.036, 0.0361. The maximum deviation from the mean of these figures is 6 per cent, and it is believed that the colorimetric estimations are accurate to within 10 per cent.

In the actual estimations the concentration of C.S.F. in the fluid diazotised never exceeded 10 per cent. It was found that 50 per cent C.S.F. made the readings about 1 per cent too high, hence the error introduced through the presence of C.S.F. is negligible. Some difficulty was experienced in determining the amount of dilution desirable before diazotisation. The following routine was elaborated: 0.2 cc. C.S.F. is made up to 3 cc. with distilled water.

(a) Take 1 cc. of this, add 1 cc. of water and diazotise as above. Take 2 cc. of resulting dye solution and dilute with water (say X cc.) until the colour is such that it can conveniently be matched against the standard colour. If now the colorimeter reading indicates m mgm. procaine in the 2 cc. test solution the procaine in the C.S.F. equals 0.75 m (X + 2) per cent.

(b) Take 1 cc. of the C.S.F. dilution, add X cc. water, diazotise 2 c. of this dilution. If the colorimeter reading and the graph indicate n mg. procaine in the 2 cc. dilute solution the C.S.F. content is 0.75 n (X + 1) per cent.

The two estimations only differ markedly when X is large. The following figures are taken from actual estimations:

X	PERCENTAGE CONCENTRATION OF PROCAINE C.S.F.		PERCENTAGE DIFFERENCE
	A	B	
100	2.98	4.7	36
90	2.53	3.75	32
40	1.18	1.46	18
20	0.76	0.81	6

Only the estimation of an early sample is therefore likely to be seriously in error, as the concentration soon falls under 1 per cent.

For the estimation of procaine in blood, 2 cc. of plasma is pipetted from 3.5 cc. of centrifuged citrated blood. To this 0.05 cc. of 33 per cent acetic acid and water up to 8 cc. are added, and the whole rapidly boiled, cooled, centrifuged and the supernatant fluid filtered. Two cubic centimeters of the clear filtrate are diazotised, using an extra 0.2 cc. phenolic reagent to develop the colour. If the colour be very pale, as it often is, instead of using the colorimeter a set of tubes containing coloured solutions from known concentrations of procaine may be prepared and used for comparison. In the diluted blood filtrate the extra phenolic reagent is necessary to overcome the acidity of the acetic acid and the buffer action of the blood salts. The method as described has been checked against blood of a normal animal to which known quantities of procaine had been added, and has been found reliable. The stability of the procaine during the boiling was also investigated. Four determinations on the same solution were carried out, before and after boiling, with the following results (milligram procaine in 2 cc. solution):

Before boiling	0.036	0.036	0.036	0.036
After boiling	0.037	0.035	0.037	0.035

These figures support Cheramy's conclusion that procaine is not destroyed by boiling for a short time in slightly acid solution. The amount of acetic acid added was the least required to give a clear filtrate.

Diazotisation of procaine in urine is complicated by the interaction of such substances as urea with the nitrous acid, which has, therefore, to be present in generous excess. The routine was modified by diluting 0.2 cc. urine to 2 cc. and using 15 per cent HCl, 16 per cent Na_2CO_3 and 16 per cent $NaNO_2$ solutions. For coupling, 0.4 cc. of the phenolic reagent is followed by 0.6 cc. of the stronger solution of Na_2CO_3. Even with these measures, the values for procaine in urine must be accepted with some reserve.

Tutocaine and larocaine can be estimated similarly, but being

stronger anaesthetics and therefore injected in smaller doses the concentrations found in blood and urine are never high, and the figures are less reliable than those for procaine.

When a local anaesthetic is injected into the C.S.F. in the lumbar region, we can consider its dilution there with C.S.F., its spread through the C.S.F. to other parts of the central nervous system, its absorption or fixation by nervous tissue, its absorption into the circulation and subsequent destruction or excretion. There is also a possibility of the formation of break-down products within the theca. It is important to remember that there are gross anatomical differences between the arrangements of the tissues within the spinal column of a tailed animal such as the cat, and man. In the cat there is no "pool" of C.S.F. below the termination of the cord. The cord in its membranes and its continuations seem to fill the bony canal. Hence any drug which is injected is less likely to be diluted rapidly than would be the case in man.

PROTOCOL

Cat: 2,600 grams, ♂, light chloralose anaesthesia

10:20	Spinal anesthetic: 50 mgm. procaine HCl in 0.5 cc., i.e., 10 per cent (blood pressure falls, 170 to 140 mm. Hg; knee-jerk −)
10:25	0.1 cc. C.S.F. collected procaine content 4.45 per cent
10:35	0.1 cc. C.S.F. collected procaine content 0.76 per cent
10:50	0.1 cc. C.S.F. collected procaine content 0.23 per cent
11:10	(Knee-jerk +). 0.1 cc. C.S.F. collected procaine content 0.04 per cent
11:30	0.1 cc. C.S.F. collected procaine content 0.02 per cent
	Urine: 10:20–10:50 3.0 cc. procaine content nil
	10:50–11:20 1.3 cc. procaine content 0.023 per cent
	11:20–12:20 1.8 cc. procaine content 0.043 per cent
	12:20–14:05 2.0 cc. procaine content 0.028 per cent
	Blood at 14:05. Procaine content nil.

If samples of C.S.F. collected at intervals through the lumbar puncture needle after administering the spinal anaesthetic be assayed for drug content as in the protocol, curves may be drawn to show the changes in concentration as time goes on. Such curves are shown in figures 1, 2, and 3 for procaine, larocaine and tutocaine respectively. The standard spinal injection for procaine which we have used is 0.5 cc. of 10 per cent solution, i.e. the

strength usually used clinically but a dose about five times the full clinical dose if body weight be taken into consideration. Less

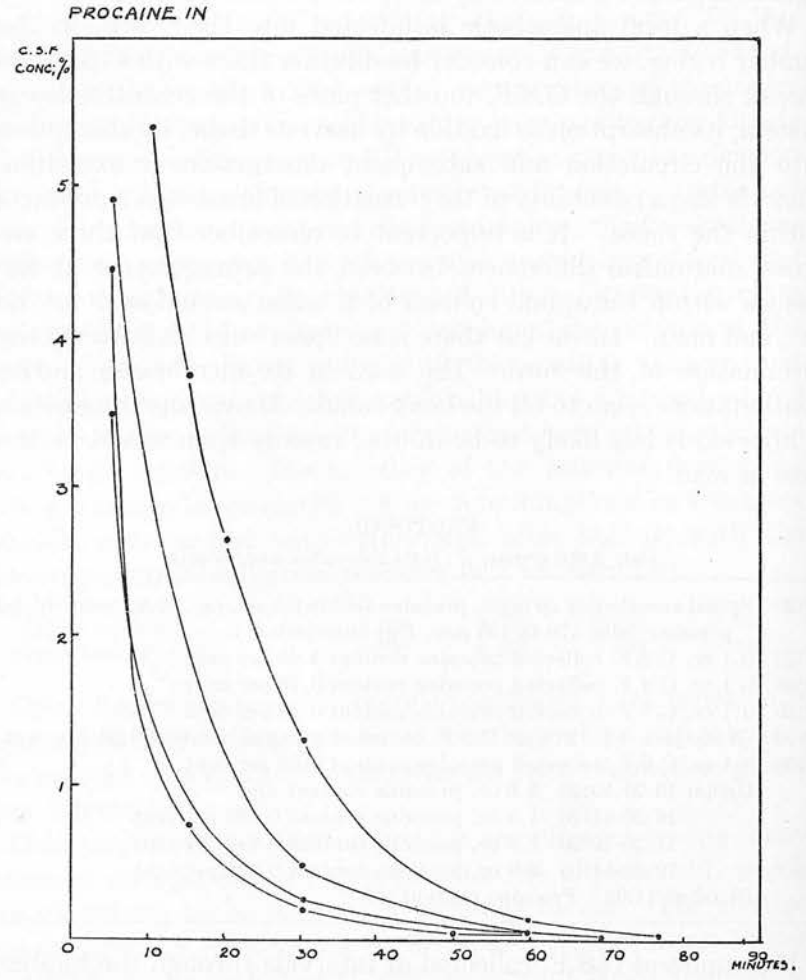

FIG. 1. PROCAINE CONCENTRATIONS IN CEREBRO-SPINAL FLUID FOLLOWING SPINAL INJECTION OF 50 MGM. PROCAINE HCl IN 0.5 CC. (10 PER CENT)

There is usually a rapid reduction in the concentration during the first 15 to 30 minutes, then more gradual. The knee-jerk returns with a concentration of 0.05 per cent procaine HCl.

concentrated solutions and smaller doses have also been used but if the volume be cut down in accordance with body weight to

0.1 cc., the precise measurement of the injection introduces difficulties. Even with this dose of 0.5 cc. the paralysis of the

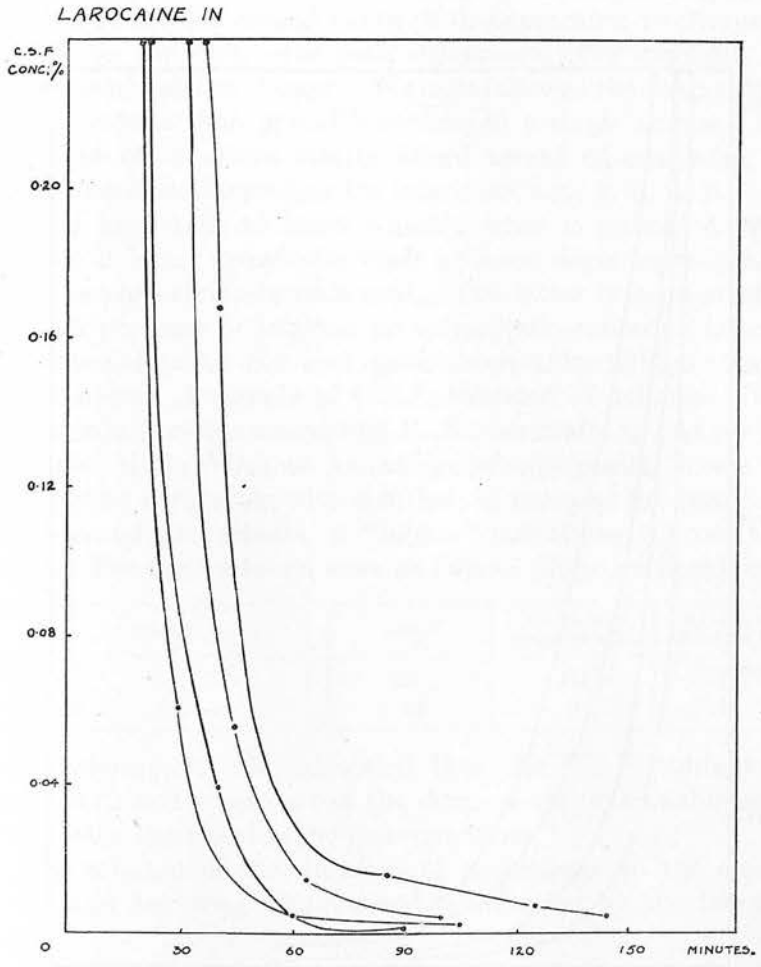

FIG. 2. LAROCAINE (INJECTED 20 MGM. IN 0.5 CC. INTRATHECALLY) CONCENTRATIONS IN CEREBRO-SPINAL FLUID
The knee-jerk returns when the concentration falls to 0.004 per cent

knee-jerk lasts as a rule for only 30 to 50 minutes—a shorter anaesthesia than the relatively smaller dose gives in man.

In such experiments as that constituting the protocol it will

be clear that the repeated removal of samples helps in the elimination. The actual amount of procaine lost in this way is only some 10 per cent of the total, and it was not usual to collect sam-

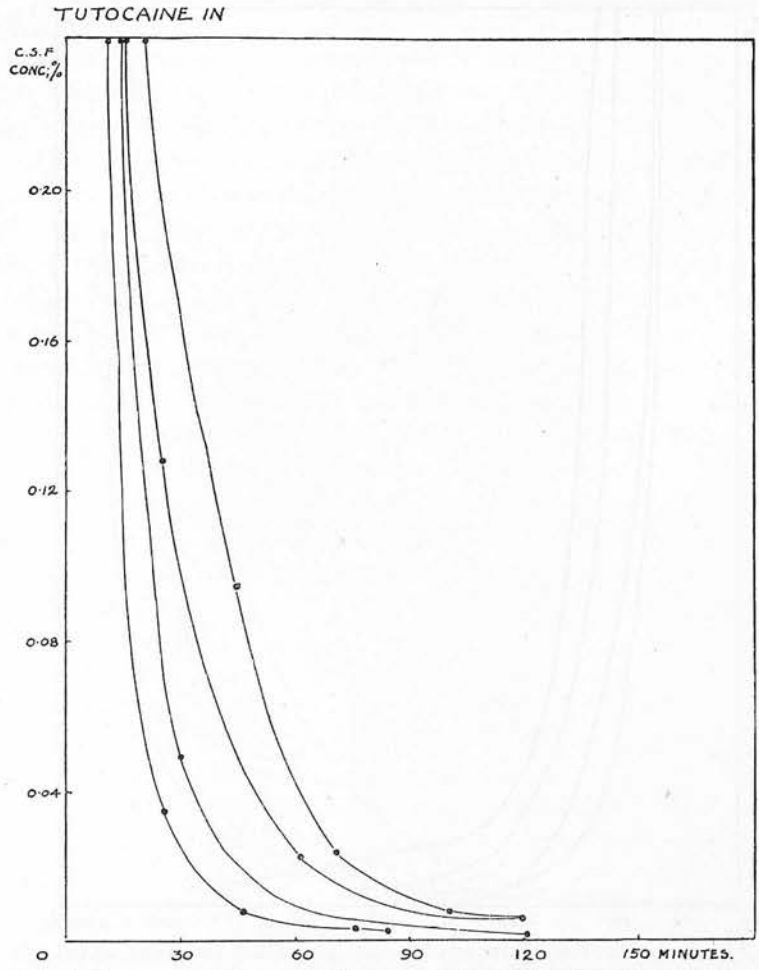

FIG. 3. TUTOCAINE (INJECTED 20 MGM. IN 0.5 CC. INTRATHECALLY) CONCENTRATIONS IN CEREBRO-SPINAL FLUID
The knee-jerk returns when the concentration falls below 0.005 per cent

ples at 5 and 15 minutes (samples relatively rich in anaesthetic) as in the protocol. Indeed if a composite graph be prepared from a number of experiments in which the C.S.F. is collected

only once during the anaesthesia, a curve similar to those of figure 1 is obtained. It has been noticed that if the spinal anaesthesia be repeated in an hour or two after the return of tendon jerks, the second curve of disappearance is always displaced to the right—the drug disappears more gradually and the anaesthesia lasts longer. We have followed the disappearance of as many as four spinal injections in a single animal. Such series of observations clearly afford means of comparing two drugs A and B, alternating the injections, e.g., A, B, A, B.

It is important to know whether what is estimated in the sample is local anaesthetic itself or some degradation product such as para-amino-benzoic acid. The latter in concentrations of 0.25 per cent or less has no anaesthetic action on injection intradermally: 0.4 per cent gave about three minutes partial anaesthesia. A sample of C.S.F. collected 15 minutes after a spinal injection was assayed by K. B. chemically as 1.14 per cent procaine HCl. Without knowledge of this result, it was also assayed by comparing with solutions of procaine for duration of intradermal anaesthesia, a "blister" containing 0.1 cc. being used. The figures found were as follows (duration in minutes):

SUBJECT	C.S.F./5	0.2 PER CENT PROCAINE HCl	0.3 PER CENT PROCAINE HCl
D. D.	12	11½	13½
A. D. M.	9½	9	12

Interpolating, it was calculated that the C.S.F. contained 5 times 0.22 or 1.1 per cent of the drug—a very reasonable agreement with the colorimetric determination.

The relation of the duration of anaesthesia to the concentration of the drug administered is indicated by the following table:

CONCENTRATION INJECTED	VOLUME INJECTED	NUMBER OF OBSERVATIONS	AVERAGE DURATION
per cent	*cc.*		*minutes*
2.5	0.5	20	17
5	0.5	20	21
10	0.5	25	35
20	0.3-0.4*	5	(53)

* According to body weight.

With 20 per cent solutions the usual dose of 0.5 cc. was fatal, inducing respiratory collapse. The dose was accordingly reduced, but no weight is attached to this figure for duration of anaesthesia because of the excessive dose and small number of observations.

SPREAD OF DRUG WITHIN THE THECA

Hill and Macdonald (1935) have emphasized that the main danger in spinal anaesthesia lies in the spread of the drug from the point of injection headwards, involving in turn the intercostal and phrenic roots and finally the respiratory centre. It was clearly interesting to know what concentration of drug was reached at the high levels following on an anaesthetic injection in the lumbar region. Samples were therefore collected at intervals from the cisterna magna in a series of cats. In most of the animals the concentration in such samples never exceeded 0.001 per cent and half this was more common; in one rather heavily chloralosed animal a concentration of 0.002 per cent was recorded an hour after the injection, and in another hour the figure was below 0.001 per cent. These figures confirm Labat's (1928) conclusion, which was not then supported, apparently, by quantitative estimations—that "specimens of C.S.F. taken in the dorsal region, higher than the level of the anaesthesia, are free from the drug or contain only very weak traces of it."

ELIMINATION IN THE URINE

We were surprised at the low concentrations in the urine and the small amount of drug excreted *via* this route. Thus in the protocol only 1.7 mgm. of procaine (3 per cent of the injected dose) were excreted by the kidneys. The highest concentration found in a sample of urine was 0.16 per cent, and in this experiment the urine accounted for about 5 per cent of the injected dose.

Similarly when tutocaine was used, in an animal which received three successive spinal injections (10, 20 and 20 mgm.) with an hour or two between injections, there was a urinary output of 38 cc. corresponding to 3.8 mgm.—under 8 per cent of the total injected.

In case the experimental animal dealt differently than man with procaine, estimations were made on the urine passed for 24 hours after 300 mgm. had been given intrathecally in man. The concentrations found were even smaller—up to 0.005 per cent—and only 10 mgm. (3 per cent of the injected dose) was excreted thus.

CONCENTRATIONS ABSORBED INTO THE BLOOD

Examinations of the blood for local anaesthetics absorbed from the theca were similarly found to account for only a small percentage of the dose. Thus two hours after induction of spinal anaesthesia the blood of the cat was always negative. The highest concentration found in the blood after a spinal injection was under 0.002 per cent. Even when procaine HCl is infused, intravenously, as in the Macdonals and Israëls (1928) method for measuring toxicity, the drug rapidly disappears from the blood. Thus an assay on a cat five minutes after an infusion of 130 mgm. in 13 minutes which temporarily paralysed respiration indicated that the total circulating blood at that time contained less than a milligram and a half of drug. More than twice this amount was separated from the 80 grams of liver.

Such figures indicate that there is no real risk of danger from blood absorption. Dunlop (1935) quotes evidence for the detoxication of procaine in the capillary bed in the dog, apart from the liver, but finds that after 15 to 20 mgm. per kilogram of procaine some persists in the blood at the end of an hour, and with repeated doses there is an accumulation in the blood of the dog of both procaine and para-amino-benzoic acid. A human check was attempted in two cases. Half-an-hour after induction, when it was considered that the blood concentration might be about a maximum, 10 cc. blood was collected from a convenient vein. In both cases the concentration was below the powers of estimation of the method i.e., under 0.002 per cent, although full doses (300 mgm.) had been given.

DISCUSSION

The rapid fall in concentration of local anaesthetic injected into the cerebro-spinal fluid in the lumbar region might be at-

tributed to dilution, fixation in the nervous tissue, spread headwards within the theca and blood absorption. On the basis of our experiments we hold the following opinions:

(1) There is no comparable dilution in the cat to what occurs in man, for anatomical reasons.
(2) There is little spread headwards, for only very low concentrations are found in the cisternal magna and it is unusual for fore-limb tendon reflexes such as the elbow-jerk to be depressed after lumbar injections.
(3) There is never any significant, still less a dangerous, concentration in the blood-stream, following absorption from the theca. Presumably the drug is removed from the blood by the liver, and to a slight extent (up to 8 per cent) by the kidneys as rapidly as it is absorbed.
(4) The drug is not broken down within the theca but absorbed in the form in which it is injected.

Since it is possible to follow the disappearance of the drug following a series of injections in a single animal, either chemically or by noting the duration of the disappearance of the knee-jerk, it is possible to compare two drugs or different concentrations of a drug as regards relative spinal anaesthetic efficiency.

SUMMARY

1. The literature dealing with the estimation of the concentration of local anaesthetics in body tissues, and the fate of such drugs when administered by intrathecal injection is reviewed.

2. A suitable technique for the estimation of local anaesthetics derived from para-amino-benzoic acid in cerebro-spinal fluid, blood and urine is described.

3. In spinal anaesthesia in the cat the concentration of drug rapidly falls at the point of injection. Little is present at any time in the blood, and little is excreted in the urine. The spread upwards from the point of injection is very limited.

4. Figures are given relating the average duration of anaesthesia with the concentration of drug injected.

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Observations on Dental Local Anæsthetic Solutions.

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INTRODUCTION.

ALTHOUGH adherents to the use of cocaine in dentistry for purposes other than surface application to mucous membranes still survive (Roper-Hall, 1935) most operators subscribe to the conclusion of the Committee of the American Medical Association—"Cocaine should never be injected." Cocaine substitutes are legion, and many manufacturers offer drugs or solutions which they praise as ideal—possessing all the desiderata possible. Such claims are by no means entirely substantiated when the preparations are subjected tc extensive pharmacological and clinical tests. There is no difficulty in evolving a product which will produce complete anæsthesia and so facilitate surgical intervention; the trouble arises in the avoidance of toxic side-actions and sequelæ.

The reactions of a patient to local anæsthetic injections may be considered under two headings—general and local. While many operators maintain that systemic reactions do not occur in their practices so long as they avoid cocaine, few who regularly observe the after-results in the mouth will deny that many injections are followed by undesirable hyperæmia after the initial ischæmia, and persistent pain or at any rate discomfort; nor is the rate of healing always as rapid as in tissues which have not been similarly exposed to infiltration with drugs which are potentially toxic. Such observations indicate that the anæsthetic rather than the surgical intervention may be responsible. It is a truism, but worthy of repetition, that the surgeon is debited with the pain he produces rather than credited with the pain he relieves (Cooley, 1936).

SYSTEMIC REACTIONS.

Most cocaine substitutes in doses such as are used in dentistry do not in themselves produce general toxic symptoms. Although cases have been recorded in which injections of relatively small amounts of such drugs have provoked grave symptoms, these injections have probably been complicated by the addition of a much more toxic agent—adrenalin—which, while it limits the anæsthetic to infiltrated tissues and reduces the rate of absorption and hence any possible toxicity of the anæsthetic base, is liable in itself to elicit circulatory disturbances. Such disturbances may be serious or merely unpleasant; they depend in part upon the vascularity of the injected tissue. An intravenous injection of even a trace of adrenalin (about 0·1 to 0·2 mg.—a dose which may occasionally be exceeded in a dental injection) may be alarming

in patients with susceptible hearts (Hume, 1927). The comparative safety of the anæsthetic drug itself has recently been strikingly demonstrated by the intravenous injection in man of full spinal anæsthetic doses of percaine (Sebrechts, 1934) and procaine (Falkner Hill, 1937).

It may well be, then, that the avoidance of systemic reactions depends upon the possibility of dispensing with the commonly added adrenalin. Since the functions of the adrenalin are to localise the anæsthetic drug and prolong and intensify its effects, the obvious ways to dispense with it are to substitute less toxic vasoconstrictors or to increase the concentration of the base in the hope that adrenalin will then become unnecessary.

Of drugs other than adrenalin used for localising the anæsthetic, cobefrin, ephedrine and post-pituitary extracts have received some attention. Ephedrine (in our experience) has nothing, and post-pituitary extracts little, of the action of adrenalin in local anæsthetic solution (Table I). Cobefrin can replace

TABLE I.—ACTION ON DURATION OF ANÆSTHESIA OF VASOCONSTRICTORS.
ANÆSTHETIC USED - 2 PER CENT. PROCAINE HYDROCHLORIDE, 1 C.C.

Injection	pH	Average duration
Anæsthetic alone	5·8	48 minutes
,, + Adrenalin, 1 : 50,000 ...	5·4	383 ,,
,, + Cobefrin, 1 : 10,000 ...	3·5	145 ,,
,, + Post-pituitary extract ½ unit	3·8	186 ,,
,, + Ephedrin, 1 : 4,000 ...	5·8	45 ,,

adrenalin. But while less toxic in its reactions in the same dose as adrenalin, it has to be used in much more concentrated solutions to be effective. Thus while adrenalin in a concentration of 1 : 75,000 is adequate for most purposes, cobefrin is usually used in a concentration of 1 : 10,000. In such concentrations increase in the pulse-rate and cardiac irregularities with blanching in the face and extremities have been observed not less frequently than with adrenalin. Enthusiastic users of cobefrin (Riethmuller, 1937) claim that in the usual concentration of 1 : 10,000 cobefrin is 25 per cent. less toxic than adrenalin 1 : 50,000. Presumably then cobefrin 1 : 10,000 is more toxic than adrenalin in concentrations below 1 : 65,000. Although weight for weight cobefrin is less toxic than adrenalin any apparent advantage is offset by the greater concentration in which it must be used.

Attempts have been made, on the analogy of spinal anæsthetic solutions, to substitute a viscous solution prepared with gum arabic or arabic acid for the ordinary adrenalin-containing solution. In 1911 Erhardt used the arabinates of cocaine, tropacocaine and novocain in lumbar anæsthesia. He considered that such salts were superior to the hydrochlorides as they were less irritant, and gave a higher grade anæsthesia of longer duration. They were also of low toxicity. He recorded the subcutaneous injection of 1 per cent. solutions of the arabinates and remarked that the absorption was slowed resulting in a longer period of anæsthesia than is the case with the hydrochlorides.

We therefore investigated the possibility of localising the procaine at the site of injection by giving it in a solution of acacia gum, and so attempting to

avoid the necessity of adding adrenalin. At the same time, we hoped further to enhance the anæsthetic power by using neutral or alkaline solutions.

Gum acacia consists largely of the arabinates of calcium, magnesium, and potassium. When, therefore, a solution is made alkaline, particularly with sodium phosphate or sodium carbonate, the calcium is precipitated. An attempt was made to prepare a pure arabic acid by precipitating an acid solution of the gum with alcohol. Still, however, a precipitate was formed when a solution of the product containing procaine was made feebly alkaline.

Finally, success was obtained in the following way: A 20 per cent. solution of acacia gum, after autoclaving for twenty minutes at 1 atmosphere to sterilise, was treated with 0·5 per cent. sodium carbonate anhydrous and 0·2 per cent. sodium phosphate anhydrous (Na_2HPO_4). After standing a short time the precipitated matter was filtered off. The clear filtrate thus obtained was adjusted to a pH of about 5 by addition of 2N hydrochloric acid, 2 per cent. procaine hydrochloride was dissolved in the product and it was then found that the solution could be made neutral or alkaline by addition of sodium bicarbonate or sodium phosphate without any precipitation.

The addition of gum has not been found useful. The presence of gum does not affect the rate of onset or duration of the block in the frog muscle-nerve preparation, when measured in the manner described further on in this paper. Gum solutions when injected into or under the skin produce the anæsthesia, but there is discoloration and associated after-pain which are more severe than arise with adrenalin solutions—but here we are passing from general to local reactions.

It is common to attribute systemic reactions to fright or nervousness, but they occur in subjects well hardened to the method. Adrenalin injections, without doubt, will add to any feelings of nervousness or anxiety which are not unnaturally often present.

Local Reactions.

The local reactions may be divided into the immediate and the delayed. The "immediate" may be enumerated as (*a*) pain; (*b*) delayed onset of anæsthesia, (*c*) hyperæmia or oligæmia. The "delayed" local reactions involve consideration of (*d*) pain, associated with (*e*) delayed healing, and (*f*) destruction of tissue, (*g*) bleeding, (*h*) unduly prolonged anæsthesia. What part, then, may the anæsthetic play in each or any of these?

(*a*) Pain on injection may of course be attributable to clumsy technique, not forgetting a blunt needle. When the prick of even a sharp needle is feared or regarded as objectionable the site of injection may be deadened by the local application of phenol or benzocaine in alcohol, &c. It is well established, however, that efficient anæsthesia so produced is followed by local sloughing in 5 to 20 per cent. of cases (Tainter *et al.* 1936, 1937). So far as the anæsthetic solutions are concerned, most act so rapidly that no pain is experienced. With solutions of percaine, which must be on the acid side since percaine is thrown out of solution by a trace of alkali or even at neutrality, there may be very transitory pain but of considerable severity. The drugs of the para-amino-benzoic-acid series do not provoke such pain even in acid solutions, but they may produce momentary discomfort, especially when injected under pressure. It cannot be assumed that acid injections are

devoid of deleterious action because they produce no pain: the covering of the pain by the action of the local anæsthetic merely means that the reaction of the tissues does not reach consciousness. If an acid saline or Ringer solution be injected, the pain—presumably an indication of tissue trauma—may be severe. Such pain—and the action of the acid solution on the tissues which produces the pain—may be intensified and prolonged by the vaso-constrictor effects of added adrenalin. With neutral or alkaline solutions there is practically no sensation with injection, even when large volumes are used which must to some extent dissect and stretch the tissues.

(*b*) The onset of anæsthesia with infiltration is almost immediate. When a regional block is attempted, it is well known that the anæsthesia may take up to twenty minutes to develop, and most operators with experience of different solutions do not find all equally rapid in yielding the desired result. We have attempted to correlate the rate of onset with the hydrogen-ion concentration of the injected solution. While such a correlation might be established clinically provided a sufficient number of cases was available, the rigidity of the experiment is limited and its control difficult. We have, therefore, established a correlation by measuring the rate of onset of nerve block in the usual frog's sciatic-gastrocnemius preparation. 1 cm. of the nerve is immersed in 1 c.c. of 0·5 per cent. procaine solution, and the time is taken for the blocking of an impulse passed along the nerve and causing a twitch in the muscle. Two nerve-muscle preparations are available from each animal; one is used as a control at pH 7·2, the other is similarly measured at the test pH and the difference in onset-time for the blocks is expressed in terms of a percentage of the time for the onset at the control pH, 7·2. Averages have been struck for a number of experiments. These are set out in Table II, and shown graphically in the accompanying figure (fig. 1). It will be seen that nerve block is produced

TABLE II.—EFFECT OF REACTION OF ANÆSTHETIC SOLUTION ON RATE OF ONSET OF ANÆSTHESIA.

pH under investigation	Number of experiments	Average difference in the duration of onset time for nerve block expressed as percentage of that at pH 7·2
5·5	8	+ 77
6·0	12	+ 39
7·2	40	0
8·0	8	− 22
9·0	8	− 72
9·5	6	− 78

more quickly with the more alkaline of any two solutions, within our experimental range. The rate of onset of anæsthesia increases with increasing pH (i.e. diminishing acidity). While clinical observations had led one to expect some such correlation, we were surprised how constant and how well marked the relation appeared to be.

A few observations were made on the rate of recovery of nerve conduction in some of the same preparations, but precise measurements of the return of conduction after washing away the anæsthetic were not very satisfactory. Our

feeling was that as a rule the more alkaline block was the more easily removed, but of course it had had a shorter exposure to the anæsthetic.

(*c*) Hyperæmia of the tissues is common with injections other than cocaine which do not contain an added vasoconstrictor. Increased bleeding may make the explorations and manipulations of the surgeon more difficult, but free bleeding at the time of operation is probably good from the point of view of the patient: it can almost always be readily controlled, and involves the flushing of the wound with an ideal cleansing solution.

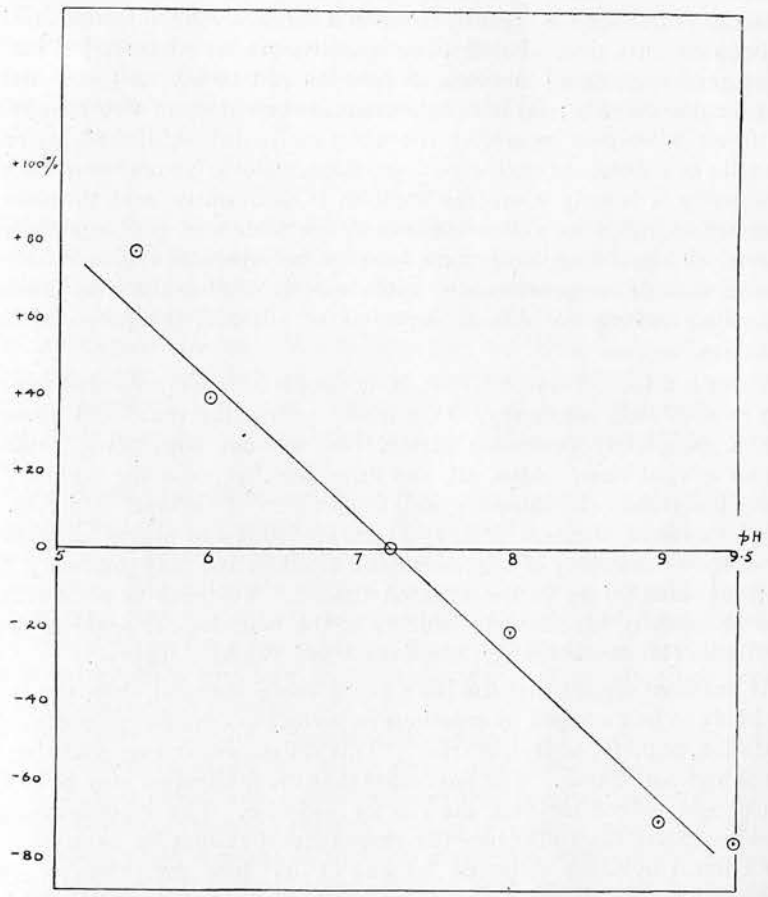

FIG. 1.—Relation of delay in onset of anæsthesia to the hydrogen-ion concentration of the solution. ORDINATE: Divergence of time for development of nerve block, expressed as percentage of that for pH 7·2. ABSCISSA: pH.

Oligæmia on the other hand makes the recognition of tissues and the operation easier. It is also known to deepen and prolong the anæsthesia. But there is a debit side to offset these credits. There is the risk of delayed hæmorrhage when the effect of the vasoconstrictor has worn off—tiresome if not dangerous; the increased risk of infection in tissues temporarily deprived of their natural defences with their circulation; the risk of devitalised tissues—suffering from the interaction of possibly prolonged infection, of anæmia, and of the toxic actions of the anæsthetic solution — causing delayed pain

and delayed healing. These delayed sequelæ may now receive some consideration.

(*d*, *e*, *f*) Delayed pain—that is to say pain which develops and persists as the effects of the local anæsthesia wear off—is not an unusual sequel. It depends, as does delayed healing, on tissue damage, and these can receive joint consideration. Although rarely severe such pain can be sufficiently unpleasant. In so far as it may be a legacy from the surgery we are not concerned with it here, but can it be due at all to the use of a local anæsthetic solution ?

We have investigated this by intra- and subcutaneous injections of similar solutions on ourselves. Briefly, our conclusions are that: (1) Pain is not experienced when small amounts of solution (0·1 to 0·5 c.c.) are introduced into or under the skin ; (2) with subcutaneous injections of 1 to 2 c.c. there is significant after-pain provided the solution is (*a*) acid, and (*b*) contains adrenalin or cobefrin in active concentration. Both factors seem necessary. Presumably it is only when the solution is sufficiently acid to cause some tissue trauma, and its action sufficiently localised and prolonged, that pain persists. To minimise the damage done by the injection, it should be neutral, isotonic, at body temperature, and contain no more adrenalin than is necessary to produce anæsthesia of such depth as to allow of the proposed surgical procedure.

There is a further reason for not using deeper or more prolonged anæsthesia than is absolutely necessary. One tends to assume that local anæsthetics have a completely reversible action, but without convincing evidence in support of that view. After all, the drug acts by poisoning normal physiological functions—the initiation and conduction of nervous impulses. Prolonged exposure of nerve endings or nerve trunks to strong solutions may reduce the reversibility of the anæsthetic's action, and may produce a neuritis or other lasting injury in the affected tissues. Evidence of such action has been abundantly produced in studies of the neurological sequelæ of spinal anæsthesia (Macdonald, 1937, Macdonald and Watkins, 1938).

It has been argued that the jaws, being more vascular than the skin, are less likely to be damaged by solutions imperfect as regards hydrogen-ion concentration, toxicity, or temperature. This difference, if any, must be one of degree and not of kind. The possibility that the infiltration may be in a septic area should indeed increase the risk of sequelæ. The injection may both spread the infection and reduce the resistance of tissues by paralysing them. The clinical evidence collected by one of us does not, however, indicate deleterious action provided neutral buffered solutions of low adrenalin content be injected, and injected without much pressure, to reduce distension of the tissues and minimise interference with the circulation.

(*g*) Delayed hæmorrhage—which may result in the surgeon being sent for in the middle of the night, by which time the patient's patience is exhausted and his anxiety and apprehension are at their height—may of course have nothing to do with the local anæsthetic. But most observers would admit that prolonged vasoconstriction is usually followed by more prolonged hyperæmia—even in the skin when the adrenalin effect wears off there is often marked dilatation of vessels and sometimes discoloration. Spasm or extreme contraction of plain muscle is frequently followed by loss of tone or even

paralysis in vessels as elsewhere. Here again, then, an unnecessarily high adrenalin content seems to be strongly contra-indicated.

(*h*) Unduly prolonged anæsthesia, apart from the above considerations, is probably also evil in that it is an unnecessary anxiety to the nervous patient. Experience shows that anæsthesia which lasts for about an hour is adequate for all practical purposes. As has already been emphasised an anæsthesia prolonged by ischæmia is undesirable. Such prolonged disturbances of sensation as have been reported following intrathecal injections are unusual within the buccal cavity, but it is a doubtful advantage to have anæsthesia lasting much longer than the working-time of the surgeon. It is reasonable to assume that the earlier the recovery from the anæsthesia, the sooner are the tissues ready to respond to normal stimuli and offer the ordinary defence of the body to injury.

Discussion.

What of the stock local anæsthetic solutions? Procaine hydrochloride is not stable in solution unless the solution be definitely acid. If hydrolysed, the resultant products have little or none of the anæsthetic action of the parent drug. If the solution is to contain adrenalin, it must also be acid to stabilise the vasoconstrictor. We believe that our indictment of acid solutions, on the grounds of rate of onset of anæsthesia and tissue trauma, is sufficient to condemn their use. When the pH of a solution lies between 3 and 4·5, as is usual with stock injections, we believe it should be rejected forthwith.

The practice of preserving sterility in many stock solutions by adding even traces of disinfectants is probably also reprehensible. The ideal antiseptic, truly specific in its actions and devoid of action on the tissues of the host, has yet to be found. The antiseptic may or may not guarantee the sterility of the product—in any case the unnecessary introduction into the tissues of even traces of drugs which are general protoplasmic poisons seems hard to justify on theoretical grounds, and on clinical evidence one of us, after fair trial, is equally opposed to such practice.

The desirability of the use of neutral solutions is, of course, no new idea. Gros (1912) established—as we have done—the more rapid action thus obtainable on frog nerve, and parallel dental evidence has been supplied by Dailey and Benedict (1929), Freeman (1929), Fischer (1933), and Wilson (1935). Cooley (1936) remarks on the slow onset of anæsthesia, the increased tissue irritation and the post-operative pain with stock solutions—stable because of a pH of 3·3. Some observers, however, seem rather dissatisfied with the obvious deductions from their own observations. Thus Gwinn and Ferber (1936) having re-established the quicker onset and quicker recovery with neutral or alkaline solutions, fear lest the duration of anæsthesia may not be adequate, and stress the disadvantages that may follow from allowing such solutions to stand, or boiling them, especially if adrenalin be present. Miller (1937), comparing stock solutions of pH 3·5 to 5·6 with freshly prepared solutions of pH 4·6 to 6·6 and solutions of procaine borate of pH 7·6 to 8·2 finds that in the mouth rate of onset of anæsthesia did not vary, nor did the post-operative results. This he attributes in part to the fact that cells do not necessarily adjust themselves to the

hydrogen-ion concentration of the medium or tissue fluid with which they are surrounded. He admits, however, that electrocardiographic studies indicate some effect from the injections on the cardiac mechanism when epinephrine is included, and has related the seriousness of such disturbances with the concentration of vasoconstrictor used. Cobefrin was found to be less toxic than epinephrine, but not without effects.

It is surprising that when the facts have been so well and variously and repeatedly established, their clinical application is so slow. The best possible technique would seem to be Fischer's dry ampoule, the salts in which are dissolved in sterile distilled water just before injection to give a neutral buffered isotonic solution of known anæsthetic and adrenalin content and rapid penetrating action. The method recommended by one of us (K.B.) for the preparation of these ampoules will be published elsewhere.

Summary.

(1) The qualities of the ideal local anæsthetic for dental use are considered.

(2) Attempts to dispense with adrenalin in such solutions are discussed.

(3) The rate of onset of anæsthesia depends upon the action of the solution : it is quicker with a neutral or alkaline than with an acid solution.

(4) It is believed that undesirable systemic and local reactions can be reduced by the use of buffered neutral solutions of procaine, in which the adrenalin content is reduced to a minimum.

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AN EXPERIMENTAL INVESTIGATION INTO THE CAUSE OF PARALYSIS FOLLOWING SPINAL ANAESTHESIA.

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British Journal of Surgery: 1938.

One of the authors has investigated a series of cases in which paralysis in the form of a cauda equina syndrome followed the employment of spinal anaesthesia in man. (Watkins and Ferguson (5)). It appeared from a study of this series that this complication was especially liable to follow the employment of a solution known as Duracaine, although occasional cases have been recorded in the literature after the use of other agents. It seemed certain that the lesion resulted from some toxic effect of the injected drug rather than from the mechanical trauma of the needle. The composition of Duracaine ("heavy") was:

Procaine HCl	"Planocaine" brand	0.1	Gram per cc.
Gliadin		0.00325	Gram per cc.
Glycerine		0.1	Gram per cc.

in 15% Ethyl Alcohol.

This was later altered to the following:

Procaine HCl	"Planocaine" brand	0.1	Gram per cc.
Gum Acacia		0.002	Gram per cc.
Glycerine		0.0416	Gram per cc.

in 15% Ethyl Alcohol.

As it was uncertain which of the constituents of Duracaine was responsible for the toxic effect, the matter was subjected to laboratory investigation, the results of which are recorded herewith.

While the actual anaesthetic agent seemed the most probable cause of the undesired symptoms, it must be remembered that

intra-thecal injections of stronger solutions of alcohol have been used for the production of prolonged anaesthesia, while glycerine is not without possible action on nervous tissue.

M E T H O D.

The animal employed in this investigation was the cat. This animal had been extensively utilised by one of the authors (A.D.M. 1) for other experimental work on spinal anaesthesia and appeared well suited to the present task. It was evident that in order to conduct this research on a practical scale, some means must be taken to render the incidence of paralysis considerably greater than that occurring in man (less than 1%). It appeared to be reasonable to bring this about by the injection of the drug in much greater relative bulk than that employed in man. Usually, the concentration injected was the same as that of the particular constituent of "heavy" Duracaine under examination, except that the alcohol and glycerine were also given in even greater concentration.

Each animal was given light ether anaesthesia after an injection of atropine sulphate gr. 1/200. A fine needle was introduced between the laminae of the sixth and seventh lumbar vertebrae, and after obtaining a good flow of cerebro-spinal fluid, 0.5 cc. of the particular fluid was injected. This dose is about five times the usual clinical dose, calculated on equivalents in terms of body weight. If this dose be exceeded, respiratory depression may follow, the drug affecting not only the intercostal but also the phrenic roots. With a

dose of 0.5 cc. the tendon jerks in the forelimb are not noticeably affected as a rule. In introducing the needle, great care is taken to minimise trauma. In particular, the needle is not pushed too far, lest it puncture the venous sinuses behind the bodies of the vertebrae. Unless a free flow of cerebro-spinal fluid is obtained, experience has shown that the injection is likely to be a failure. Similarly, if the fluid contains blood, the resulting anaesthesia may be less certain in its duration and extent.

The cat was held vertically or fixed in a reclining position with the head uppermost. This was intended to limit the spread of the anaesthetic and sustain a good concentration in contact with the lumbar roots for some time. When the injection contained procaine, the knee jerks were observed to disappear quickly and as the cat emerged from the ether anaesthesia, paralysis of the hind limbs and hind part of the body was manifest in its attempts to move about. When the solution contained only alcohol and/or glycerine, some degree of weakness was sometimes observed when the animal first recovered from the ether, but the knee jerks never disappeared.

LESION PRODUCED.

The animals were kept under observation for varying periods following the injection. In a certain number, as shown below, a typical cauda equina syndrome ensued. Thus there was paralysis of the bladder, sphincter ani and tail, sometimes weakness of the

hind limbs and also impairment of sensation. In those animals which developed paralysis, the lesion was always manifest when the animal was examined one to three days after injection.

The lesion was not always as extensive as that described above and frequently the paralysis was limited to the tail. In such cases, when the animal was stroked, the base of the tail was elevated whilst the remaining two-thirds or three-fourths hung flaccid from the base. Sometimes also, the tail would deviate to one or other side. The distal part of the tail also was insensitive to pinching and pressure.

In the complete lesion, the picture was identical with that observed by one of the authors following division of the roots of the cauda equina or excision of the conus medullaris in dogs (Watkins (6)). The bladder became very greatly distended and by firm pressure urine could be expressed in a stream which ceased instantly on release of the pressure. In an animal which was not paralysed, the bladder was never particularly large and expression was impossible. On rare occasions, compression would set up urination in a normal animal, but under such circumstances the stream continued after release of the pressure until by reflex micturition the bladder had emptied. After about three weeks, the affected bladder became very much reduced in size, but it continued to be unusually large and its contents expressible in the manner above described. The contents of the bladder in one cat, which was kept alive for six months after its spinal anaesthesia, remained easily

expressible, though at the last examination, the stream produced by expression did not seem to stop quite so abruptly on release of the pressure. In another cat, expression began to be more difficult after six weeks, but the bladder was still abnormally distended after seven months.

The paralysis of the anal sphincter showed itself by abnormal prominence of the anus, and frequently it would be seen to be widely relaxed over a scybalous mass. Impairment of sensation in the tail and in the area surrounding the anus was suggested by the extremely dirty condition of these areas in previously clean animals: and the tail was shwon to be insensitive to pinching and deep pressure. Flaccidity of the tail was demonstrated in the manner above described. In the two animals which were kept alive for a prolonged period, the tail began to show gradual recovery after six or seven weeks and could be raised to the vertical after three or four months.

R E S U L T S.

The full results may be summarized in Table I as follows:-

TABLE I.

	Total injected.	Not paralysed.	Paralysis bladder, anal sphincter and tail.	Paralysis tail only.	Total paralysed.	Percentage paralysed.
Alcohol & Glycerine	23	23	-	-	-	-
"Heavy" Duracaine	23	10	9	4	13	56
10% Procaine (Planocaine brand)	10	5	2	3	5	50
10% Procaine (Novocaine brand)	23	17	4	2	6	26
5% Stovaine (Barker)	10	4	-	6	6	60

These results appear to be conclusive proof that the toxic agent which is responsible for the paralysis after Duracaine is the procaine itself. That other anaesthetic agents besides procaine may produce paralysis is indicated by the experiments in which Stovaine (Barker) was employed. It is not suggested that the above figures establish any difference between the brands of procaine.

The failure to produce a single case of paralysis following the employment of alcohol and glycerine (frequently in higher concentration than that in "heavy" Duracaine itself) serves to show that they are not directly responsible for the lesion and still further disposes of the view that the condition results from trauma by the point of the needle. In one animal in which extensive trauma had occurred in repeated unsuccessful attempts to obtain a flow of cerebro-spinal fluid, no injection was made, nor did any paralysis subsequently develop.

Further evidence is provided in the same direction by a series of experiments in which have been correlated the incidence of sequelae

with the concentration of local anaesthetic used. In a series of 20 cats injected with 0.5 cc of 2.5 per cent procaine no prolonged paralysis was seen. In a further 20 treated with 5 per cent procaine two showed pronounced tail droop, but bladder and rectum symptoms did not arise. From the earlier table we conclude that in 56 cats injected with 10 per cent solutions sequelae appear in over forty per cent. A few were then given 20 per cent procaine. In the first two, with 0.5 cc of solution, this dosage proved too much for respiration, in six others, therefore, 0.3 or 0.4 cc was given, according to body weight. One more died acutely, leaving five survivals, of which four developed severe lesions, affecting tail, rectum, bladder and even the hind legs. (See Table 2.)

TABLE 2.

RELATION OF DOSAGE TO INCIDENCE OF SYMPTOMS.

(PROCAINE HCl).

Concentration (per cent)	Number of Animals.	Number showing some PARALYSIS.	Percentage Paralysis.
2.5	20	0	0
5	20	2	10
10	56	24	43
20	8	4	(80)+

+ (3 died acutely).

As in the case of man, the ultimate paralysis seemed to be localised to the terminal spinal nerves, although the injected anaesthetic agent caused a much more widespread paralysis of 30 to 60 minutes duration. This implies that the agent only causes lasting paralysis of those nerves which are bathed by the substance in its full concentration. The nerves which it reaches after dilution with cerebro-spinal fluid recover their function rapidly - as soon as the agent is absorbed or destroyed. Because of the paralysing effect of the drug in its full concentration of 10%, the injection of cats with a relatively greater bulk determined a much higher incidence of paralysis in these than in man.

It is of special interest to observe that paralysis was found to follow the use of stovaine as well as of procaine, the active constituent of Duracaine. It seems certain that the incidence of paralysis following the use of stovaine in man is small, very few cases having been recorded in the literature. It is doubtful whether in this investigation "heavy" Duracaine is more damaging than the preparations of procaine alone, and the question remains as to whether or not the addition of alcohol and glycerine (innocuous of themselves) enhances the toxic action of the procaine itself.

DISCUSSION:

It has long been held that the intra-thecal injection of spinal anaesthetics in high concentrations, leads to toxic

changes in the spinal cord and spinal roots both in animals and in man. (van Lier (3) 1907, Spielmeyer (4) 1908, Wossidlo (7) 1908). Spielmeyer also observed paralysis of the hind limbs and diminished tendon jerks in a dog after the intra-thecal injection of 0.04 grams of stovaine. In 1933, Lundy, Essex and Kernohan (2) showed that the intra-thecal injection of 5 cc. of 20% procaine caused extensive paralysis in dogs. These workers however, did not find paralysis from solutions of 17.5% or less, nor, surprisingly enough, was paralysis noticed in dogs injected with only 2.5 cc. of 50% solution.

It appears certain from the present investigation that the spinal anaesthetic agents in the concentrations ordinarily employed in man are toxic to nerve tissues to a degree capable of causing serious lasting paralysis in animals. In man, their rapid dilution with cerebro-spinal fluid is sufficient, except in rare instances, to prevent such paralysis, and in any event determines that when such paralysis does occur it is of relatively limited extent. In the experimental animal, the bony canal is much more filled by nervous tissue and there is only a small amount of cerebrospinal fluid wherewith the injection can be diluted.

It would seem reasonable to suppose that the risk of paralysis would be considerably reduced in practice if the anaesthetic agents were injected in less concentrated form. Indeed, Lundy, Essex and Kernohan (2) state that following a temporary paralysis from the use of 10% procaine in man, they

"have avoided the use of solutions stronger than 7 or 8% and commonly employ concentrations of 3, 4 or 5% solutions as it leaves the syringe".

On the other hand, the volume of the solution cannot be indefinitely increased without the introduction of the risk of respiratory depression. If, however, the usual 2 to 3 ccs of 10% procaine (a dose, incidentally, which may be double the maximum recommended by the British Pharmacopoeia, 1932 (150mg.)) is first diluted with cerebrospinal fluid and injected slowly so that dilution time is available, delayed toxic symptoms should be very rare.

An attempt was made to investigate the histological changes induced in the spinal roots in prolonged paralysis, but the difficulties of interpretation are such that at present no useful contribution to this aspect of the problem can be offered, though its importance is fully appreciated.

S U M M A R Y.

Intra-thecal injections of spinal anaesthetic solutions and the various constituents of these solutions into a series of about a hundred and fifty cats have shown:

1. that certain cocaine substitutes, in concentrations commonly employed clinically, though in relatively larger doses, may produce lasting symptoms comparable to caudal lesions.

2. that improvement in these cases is usually apparent in some six weeks, but cure may be incomplete in six months.

3. that the common constituents of the solution other than the local anaesthetic drug do not of themselves produce lasting paralysis in the concentrations in which they are normally employed.

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AN EXPERIMENTAL INVESTIGATION INTO THE CAUSE OF PARALYSIS FOLLOWING SPINAL ANÆSTHESIA

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EXPERIMENTAL SURGERY

AN EXPERIMENTAL INVESTIGATION INTO THE CAUSE OF PARALYSIS FOLLOWING SPINAL ANÆSTHESIA*

BY A. D. MACDONALD AND K. H. WATKINS

ONE of the authors has investigated a series of cases in which paralysis in the form of a cauda equina syndrome followed the employment of spinal anæsthesia in man (Ferguson and Watkins[5]). It appeared from a study of this series that the complication was especially liable to follow the employment of a solution known as duracaine, although occasional cases have been recorded in the literature after the use of other agents. It seemed certain that the lesion resulted from some toxic effect of the injected drug rather than from the mechanical trauma of the needle. The composition of duracaine ('heavy') was:—

Procaine HCl, 'Planocaine' brand ..	0·1 g. per c.c.
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In 15 per cent ethyl alcohol.

This was later altered to the following:—

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As it was uncertain which of the constituents of duracaine was responsible for the toxic effect, the matter was subjected to laboratory investigation, the results of which are recorded below.

While the actual anæsthetic agent seemed the most probable cause of the undesired symptoms, it must be remembered that intrathecal injections of stronger solutions of alcohol have been used for the production of prolonged anæsthesia, while glycerin is not without possible action on nervous tissue.

METHOD OF EXPERIMENT

The animal employed in this investigation was the cat. This animal had been extensively utilized by one of the authors (A. D. M.[1]) for other experimental work on spinal anæsthesia, and appeared well suited to the present task. It was evident that in order to conduct this research on a practical scale, some means must be taken to render the incidence of paralysis considerably greater than that occurring in man (less than 1 per cent). It appeared to be reasonable to bring this about by the injection of the drug in much greater relative bulk than that employed in man. Usually the concentration injected was the same as that of the particular constituent

* From the Department of Pharmacology, University of Manchester.

of ' heavy ' duracaine under examination, except that the alcohol and glycerin were also given in even greater concentration.

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The cat was held vertically or fixed in a reclining position with the head uppermost. This was intended to limit the spread of the anæsthetic and sustain a good concentration in contact with the lumbar roots for some time. When the injection contained procaine, the knee-jerks were observed to disappear quickly, and as the cat emerged from the ether anæsthesia, paralysis of the hind limbs and hind parts of the body was manifest in its attempt to move about. When the solution contained only alcohol and/or glycerin, some degree of weakness was sometimes observed when the animal first recovered from the ether, but the knee-jerks never disappeared.

Lesion Produced.—The animals were kept under observation for varying periods following the injection. In a certain number, as shown below, a typical cauda equina syndrome ensued. Thus there was paralysis of the bladder, sphincter ani, and tail; sometimes weakness of the hind limbs, and also impairment of sensation. In those animals which developed paralysis, the lesion was always manifest when the animal was examined one to three days after injection.

The lesion was not always as extensive as that described above, and frequently the paralysis was limited to the tail. In such cases, when the animal was stroked, the base of the tail was elevated whilst the remaining two-thirds or three-fourths hung flaccid from the base. Sometimes also, the tail would deviate to one or other side. The distal part of the tail also was insensitive to pinching and pressure.

In the complete lesion, the picture was identical with that observed by one of the authors following division of the roots of the cauda equina or excision of the conus medullaris in dogs (Watkins[6]). The bladder became very greatly distended, and by firm pressure urine could be expressed in a stream which ceased instantly on release of the pressure. In an animal which was not paralysed, the bladder was never particularly large and expression was impossible. On rare occasions compression would set up urination in a normal animal, but under such circumstances the stream continued after release of the pressure until by reflex micturition the bladder had emptied. After about three weeks the affected bladder became very much reduced in size, but it continued to be unusually large and its contents expressible in the manner above described. The contents of the bladder in one cat, which was kept alive for six months after its spinal anæsthesia, remained easily expressible, though at the last examination the stream produced by expression did not seem to stop quite so abruptly on release of the pressure. In another cat, expression began

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RESULTS

The full results may be summarized in *Table I* as follows:—

Table I.—EXPERIMENTAL RESULTS

	TOTAL INJECTED	NOT PARALYSED	PARALYSIS OF BLADDER, ANAL SPHINCTER, AND TAIL	PARALYSIS OF TAIL ONLY	TOTAL PARALYSED	PERCENTAGE PARALYSED
Alcohol and glycerin	23	23	–	–	–	–
'Heavy' duracaine	23	10	9	4	13	56
10 per cent procaine (Planocaine brand)	10	5	2	3	5	50
10 per cent procaine (Novocain brand)	23	17	4	2	6	26
5 per cent stovaine (Barker)	10	4	–	6	6	60

These results appear to be conclusive proof that the toxic agent which is responsible for the paralysis after duracaine is the procaine itself. That other anæsthetic agents besides procaine may produce paralysis is indicated by the experiments in which stovaine (Barker) was employed. It is not suggested that the above figures establish any difference between the brands of procaine.

The failure to produce a single case of paralysis following the employment of alcohol and glycerin (frequently in higher concentration than that in 'heavy' duracaine itself) serves to show that they are not directly responsible for the lesion, and still further disposes of the view that the condition results from trauma by the point of the needle. In one animal in which extensive trauma had occurred in repeated unsuccessful attempts to obtain a flow of cerebrospinal fluid, no injection was made, nor did any paralysis subsequently develop.

Further evidence is provided in the same direction by a series of experiments in which have been correlated the incidence of sequelæ with the concentration of local anæsthetic used. In a series of 20 cats injected with 0·5 c.c. of 2·5 per cent procaine no prolonged paralysis was seen. In a further 20 treated with 5 per cent

procaine 2 showed pronounced tail droop, but bladder and rectal symptoms did not arise. From the earlier table we conclude that in 56 cats injected with 10 per cent solutions sequelæ appear in over 40 per cent. A few were then given 20 per cent procaine. In the first 2, with 0·5 c.c. of solution, this dosage proved too much for respiration; in 6 others, therefore, 0·3 or 0·4 c.c. was given, according to body weight. One more died acutely, leaving 5 survivals, of which 4 developed severe lesions, affecting tail, rectum, bladder, and even the hind legs. (*See Table II.*)

Table II.—Relation of Dosage of Procaine HCl to Incidence of Symptoms

Percentage Concentration	Number of Animals	Number showing some Paralysis	Percentage Paralysis
2·5	20	0	0
5	20	2	10
10	56	24	43
20	8	4	80*

* 3 died acutely.

As in the case of man, the ultimate paralysis seemed to be localized to the terminal spinal nerves, although the injected anæsthetic agent caused a much more widespread paralysis of thirty to sixty minutes' duration. This implies that the agent only causes lasting paralysis of those nerves which are bathed by the substance in its full concentration. The nerves which it reaches after dilution with cerebro-spinal fluid recover their function rapidly—as soon as the agent is absorbed or destroyed. Because of the paralysing effect of the drug in its full concentration of 10 per cent, the injection of cats with a relatively greater bulk determined a much higher incidence of paralysis in these than in man.

It is of special interest to observe that paralysis was found to follow the use of stovaine as well as of procaine, the active constituent of duracaine. It seems certain that the incidence of paralysis following the use of stovaine in man is small, very few cases having been recorded in the literature. It is doubtful whether in this investigation 'heavy' duracaine is more damaging than the preparations of procaine alone, and the question remains as to whether or not the addition of alcohol and glycerin (innocuous in themselves) enhances the toxic action of the procaine itself.

DISCUSSION

It has long been held that the intrathecal injection of spinal anæsthetics in high concentrations leads to toxic changes in the spinal cord and spinal roots, both in animals and in man (van Lier,[3] 1907; Spielmeyer,[4] 1908; Wossidlo,[7] 1908). Spielmeyer also observed paralysis of the hind limbs and diminished tendon-jerks in a dog after the intrathecal injection of 0·04 g. of stovaine. In 1933, Lundy, Essex, and Kernohan[2] showed that the intrathecal injection of 5 c.c. of 20 per cent procaine caused extensive paralysis in dogs. These workers, however, did not find paralysis from solutions of 17·5 per cent or less, nor, surprisingly enough, was paralysis noticed in dogs injected with only 2·5 c.c. of 50 per cent solution.

It appears certain from the present investigation that the spinal anæsthetic agents in the concentrations ordinarily employed in man are toxic to nerve tissues to a degree capable of causing serious lasting paralysis in animals. In man their rapid dilution with cerebrospinal fluid is sufficient, except in rare instances, to prevent such paralysis, and in any event determines that when such paralysis does occur it is of relatively limited extent. In the experimental animal the bony canal is much more filled by nervous tissue, and there is only a small amount of cerebrospinal fluid wherewith the injection can be diluted.

It would seem reasonable to suppose that the risk of paralysis would be considerably reduced in practice if the anæsthetic agents were injected in less concentrated form. Indeed, Lundy, Essex, and Kernohan[2] state that following a temporary paralysis from the use of 10 per cent procaine in man, they " have avoided the use of solutions stronger than 7 or 8 per cent, and commonly employ concentrations of 3, 4, or 5 per cent solutions as it leaves the syringe ".

On the other hand, the volume of the solution cannot be indefinitely increased without the introduction of the risk of respiratory depression. If, however, the usual 2 to 3 c.c. of 10 per cent procaine—a dose, incidentally, which may be double the maximum recommended by the British Pharmacopœia, 1932 (150 mg.)—is first diluted with cerebrospinal fluid and injected slowly so that dilution time is available, delayed toxic symptoms should be rare.

An attempt was made to investigate the histological changes induced in the spinal roots in prolonged paralysis, but the difficulties of interpretation are such that at present no useful contribution to this aspect of the problem can be offered, though its importance is fully appreciated.

SUMMARY

Intrathecal injections of spinal anæsthetic solutions and the various constituents of these solutions into a series of about 150 cats have shown :—

1. That certain cocaine substitutes, in concentrations commonly employed clinically, though in relatively larger doses, may produce lasting paralysis comparable to lesions of the cauda equina.

2. That improvement in these cases is usually apparent in some six weeks, but cure may be incomplete in six months.

3. That the common constituents of the solution other than the local anæsthetic drug do not of themselves produce lasting paralysis in the concentrations in which they are normally employed.

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ACTION OF ADRENALINE ON THE PERFUSED FISH HEART.

By A. D. Macdonald. From the Marine Biological Laboratory, Plymouth, and the Physiology Department, University of Edinburgh.

(*Received for publication* 30*th September* 1924.)

CONTENTS.

Introduction.

While the action of adrenaline on the perfused amphibian and mammalian heart has been very fully studied, its action on other vertebrate hearts has received rather cursory attention, and there is some difficulty in reconciling the sympathomimetic theory of Elliot (5), applied by him to the hearts of the reptile, bird, amphibian, and mammal, with the effects obtained on the hearts of the invertebrates Maia and Pecten by Hogben and Hobson (6), where there is no known equivalent of a sympathetic innervation. There is general agreement concerning the effects of adrenaline on the mammalian heart; divergent accounts have been given of the frog, and in the case of the avine and reptilian ventricle its influence has been denied. There exists, to the writer's knowledge, no data bearing on the heart of the fish, and the present investigation was undertaken in the hope of shedding some light on the discrepancies with regard to the observations in the various classes of land vertebrates. The results were somewhat unexpected, and it became necessary to extend the scope of the inquiry, repeating earlier work on the relation of the fish heart to electrolytes, supplemented by experiments on the action of certain drugs, so that the conditions appropriate to the observations might be defined with all possible precision.

The dogfish (Scyllium) has been used, the specimens averaging some 30 cm. in length. A few experiments have been made on rays, with quite comparable results. The fish, freshly pithed, was opened ventrally, and the heart exposed by cutting away part of the pectoral girdle.

The cardinal sinuses were tied off, and perfusion carried out from the hepatic sinus, the aorta being cut across or cannulated to afford a ready egress, well away from the heart. By perfusing from a small reservoir with an overflow tube, which could be kept filled from stocks of control or experimental solutions, the pressure head was kept quite constant, usually about 10 cm. A few hearts were perfused from the aorta, but this was less generally satisfactory. The stock perfusion solution was Mines' modification of Knowlton's fluid :—

NaCl	M/2	440 c.c.
KCl	,,	14 ,,
$CaCl_2$	,,	8 ,,
$MgCl_2$	,,	10 ,,
Urea, 10 per cent.		200 ,,
Distilled water		to 1000 ,,

The half-molar solutions were prepared by weight, checked by titration, and adjusted. (In the later experiments the magnesium chloride was omitted, as it seemed to be unnecessary.) Under these conditions the heart beats freely for many hours, and indeed continues to beat long after perfusion has been discontinued, yet is quickly sensitive to changes in the medium, other than in $MgCl_2$ content below a certain limit.

The temperature at which these experiments were carried out was that of the room—about 17° C. Stock solutions were kept at that temperature for at least twenty-four hours before use. Apart from a thorough agitation in the course of preparation, no aeration of the medium was used, nor seemed in the least necessary. To guard against error arising from comparison of observations at slightly different temperatures, the variation of the heart-beat with temperature was recorded by perfusing hearts into whose ventricle a small fine thermometer was inserted through the aorta. The heart stopped at 4° C., began again at 8° C., and accelerated steadily up to 29° C., at which the contractions stopped. The amplitude did not change appreciably between 8° and 21° C., but at the latter temperature there was an abrupt increase, and thereafter no appreciable change until it was abolished at the upper limit.

Experiments on the Action of Electrolytes.

The inhibitory component of the adrenaline response, to be described shortly, made it desirable to re-examine the relation of the constituents of the normal perfusion fluid to cardiac inhibition. As magnesium proved an unessential and relatively inactive ingredient, attention was chiefly directed to the limiting concentrations of potassium and hydrogen ion. At the same time Mines' observations on the di- and trivalent

ions (8) were confirmed and extended by comparison with the effect of barium, as described below. It may be noted that no difference in sensitivity to the ions of lanthanum and cerium such as MINES' correlated with the limiting cH· for the hearts of the ray and the dogfish could be demonstrated in these experiments. Moreover, such correspondence would not be anticipated from the standpoint of the more recent work on membrane potential (LOEB). LOEB (7) has suggested that trivalent ions exert their influence on gelatine films through the formation of complex ions, and draws attention to the fact that their effects are much less rapidly reversed than those of the hydrogen ion itself. It is therefore of interest to note that the influence of $La^{\cdot\cdot\cdot}$ and $Ce^{\cdot\cdot\cdot}$ in liminal concentration is of longer duration than that of H· when the heart is again perfused with the control solution.

The threshold for inhibition in the case of K· depends, as is clear from the following observations, upon the amount of this ion with which the heart is already in equilibrium.

(*a*) Any small increase of KCl in the perfusing fluid at once produces slowing, with rapid recovery of the normal beat. Conversely any small decrease sets up acceleration, with incomplete relaxation; but if the heart is then supplied with the control medium the same initial inhibition appears as on perfusion with an excess of K·.

(*b*) On perfusing with NaCl, or NaCl and urea alone, the beat falls off rapidly, and if at this stage, *i.e.* before complete stoppage is attained, the normal medium is restored, the heart-beat stops abruptly and completely, and only after an interval begins to regain its tone and rhythm. This failure can readily be shown to be due to the potassium constituent of the control fluid.

If, as MINES supposed, potassium exerts its effect by actually entering the cell, it might well be believed that these results are due to the higher migration velocity of K·, since this would confer precedence over antagonising ions in its *initial* effects, on bringing about any change in the ionic equilibrium of the cell.

As regards changes in cH, the heart is arrested at pH 5·8 (fig. 2), the beats becoming progressively slower without loss of amplitude and ceasing as a rule in less than two minutes. The stock solution has a pH of 6·9. Between 6·1 and 5·9 the heart is slowed, and it will be seen from fig. 3 (upper half) that in a heart in which beats are grouped, alternating in origin between the sinus (slow), and bulbus (fast), reduction of the pH from 6·6 to 5·9 results in the beats becoming purely "sinus," ventricular systole being preceded by a well-marked auricular contraction. This suggests that the sinus, as an originator of contraction, is less susceptible to increase in cH than the bulbus. As regards the upper pH limit, it lies above the range for which indicators were available, and a change from pH 7·0 to 9·4 had but little apparent action on the heart. The further addition of alkali causes a rapid shortening

of the beat and arrest in systole. In these experiments and in all where changes in pH were under observation the solutions were adequately buffered and repeatedly tested by indicators.

MINES omitted to test the action of barium. Its action seems

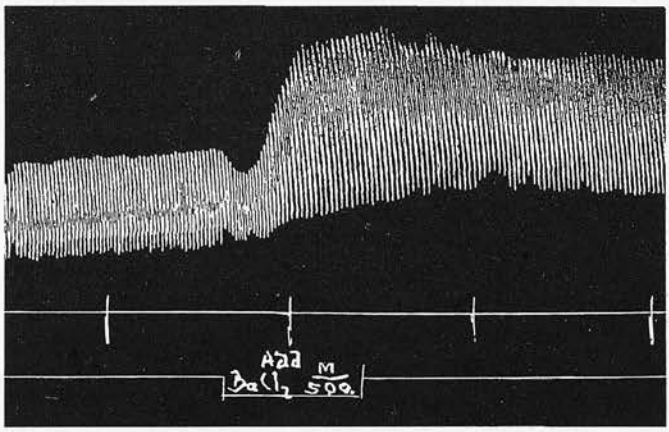

FIG. 1.—Addition to perfusing fluid of $BaCl_2$, M/500. All time-signals in minutes. All tracings of heart of Scyllium. All tracings to be read from left to right.

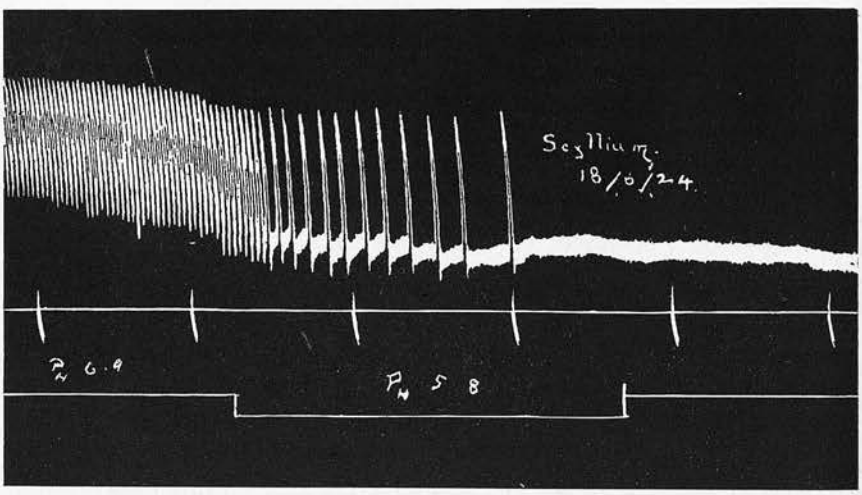

FIG. 2.—pH of medium changed from 6·9 to 5·8 at signal.

similar to that of calcium, which it can at any rate temporarily replace. The addition to stock solution of $BaCl_2$ to give a concentration of M/1000 Ba gives an appreciable increase in tone and amplitude. The effect of M/500 is shown in fig. 1. M/100 causes large, slow, irregular beats. M/50 stops the heart in extreme contraction.

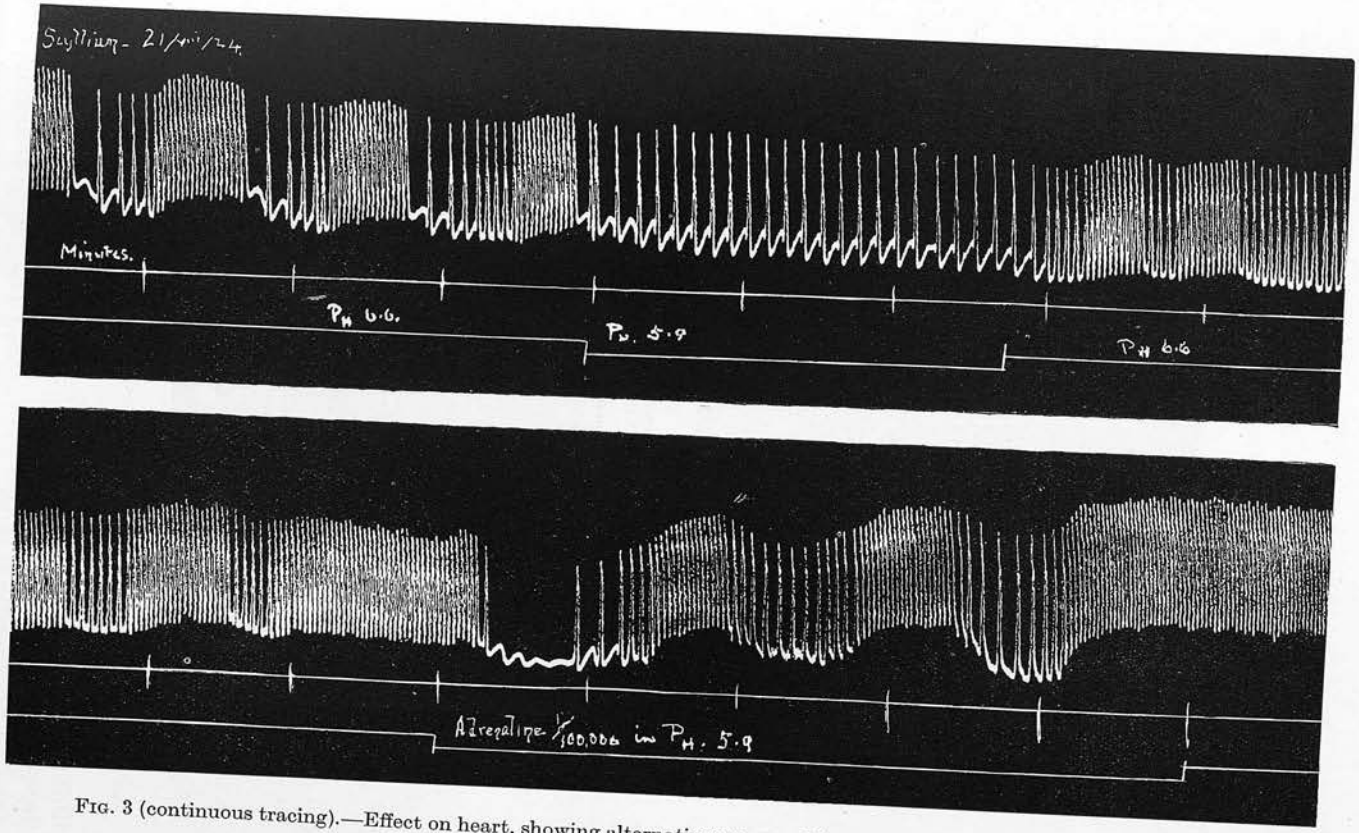

FIG. 3 (continuous tracing).—Effect on heart, showing alternating groups of beats as result of change from pH 6·6 to 5·9, and response to adrenaline, 1/100,000, in medium pH 5·9.

The Inhibitory Mechanism.

Because of the inhibitory component of the response to adrenaline it seemed advisable to examine, in case of any interrelationship, the

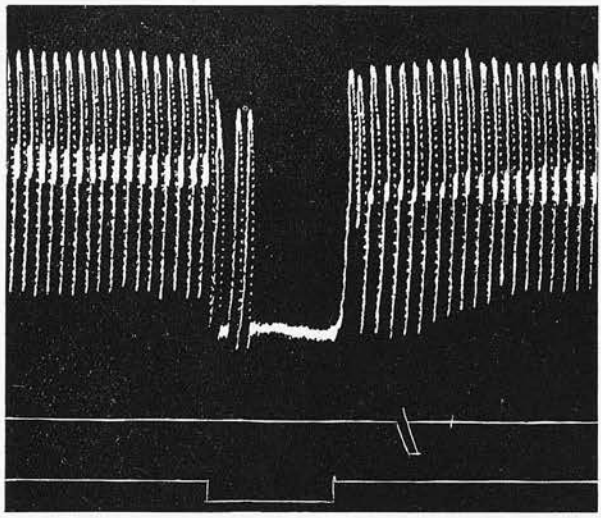

Fig. 4.—Strong stimulation of vagus nerve at signal.

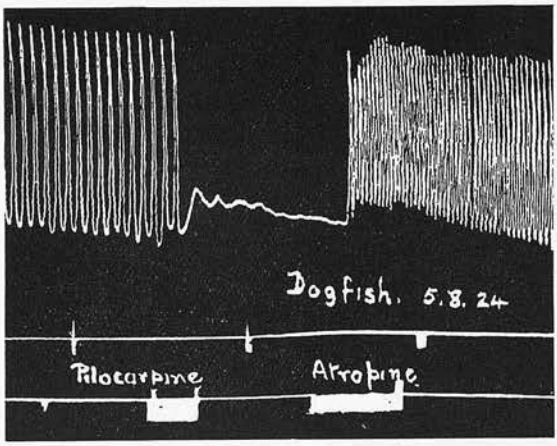

Fig. 5.—Action of pilocarpine, 1/10,000, and antagonism by atropine, 1/10,000.

inhibitory mechanism. The following observations were therefore made :—

(*a*) Vagus stimulation (either nerve, near the brain) if strong, stops the heart promptly in diastole (fig. 4). Recovery is complete and immediate on stopping the stimulation. A weaker stimulus may merely slow

the heart, with some loss of tone. An isolated heart-vagus preparation can readily be made from dogfish or ray; it is hoped at some future date to study the effect of changes in the perfusion fluid on the response to vagal stimulation.

(*b*) Pilocarpine and physostigmine added to the perfusion fluid act in the same way as stimulation of the vagus, and their action, too, can be prevented or destroyed by atropine (fig. 5). The fish heart is slowed by pilocarpine nitrate in a concentration of 1 in 500,000, stopped by 1 in 100,000, but recovers in a few minutes in stock solution. Physostigmine salicylate in the latter concentration stops the heart permanently.

Adrenaline Experiments.

The effects of the addition of small quantities of adrenaline to the perfusing fluid seem at first sight rather complicated. In fig. 6 is shown

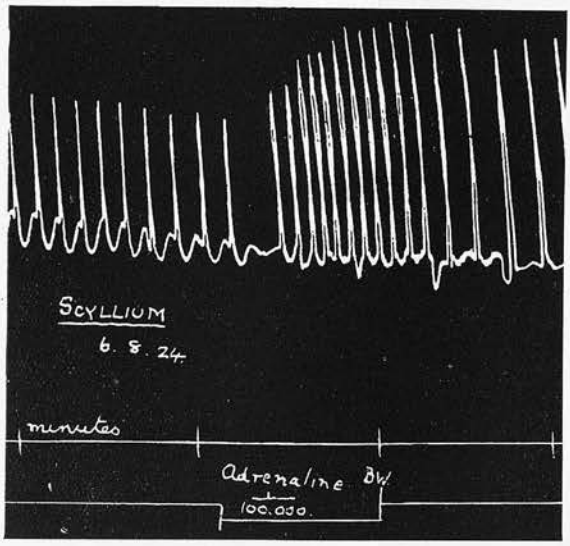

Fig. 6.—Response of heart to adrenaline, 1/100,000 at signal.

the action of 1 in 100,000 adrenaline on a slowly beating heart (seven beats a minute). There is a stoppage for twenty seconds, then a marked gain in amplitude (50 per cent.) and acceleration (twelve beats a minute). The perfusion lasted about a minute. Thereafter the rate rapidly and the amplitude more slowly return to normal. This may be regarded as the typical adrenaline effect on the fish heart. If the heart be beating rapidly there may be no gain in rate, or indeed an actual slowing (fig. 3, lower half). If the initial dose be large enough to produce a well-marked response, subsequent doses give no initial inhibition, and no appreciable change in rate, although some amplitude effect persists.

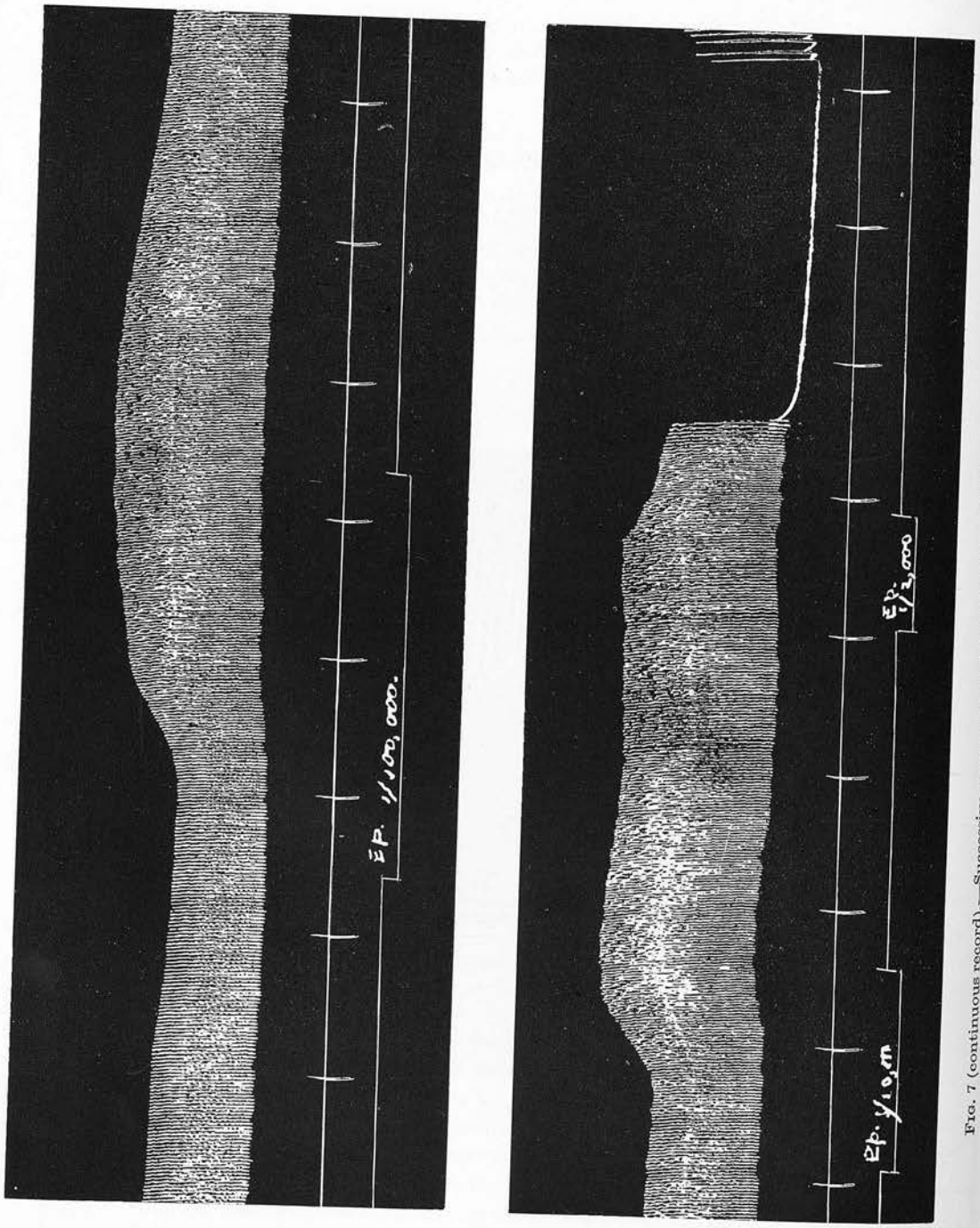

FIG. 7 (continuous record).—Successive responses to epinine in dilutions 1/100,000, 1/10,000, 1/2000; 1/10,000,000, and 1/1,000,000 had been administered previously.

In studying these phenomena a good dose for standard use is 1 mg. in 100 c.c. (1 in 100,000) of the " soloid " product of Burroughs Wellcome, but an amplitude effect can be obtained with 1 in 5,000,000. The minimal active dose of " epinine," the synthetic product of the same firm, is of the same order ; certainly its activity, as far as this response is concerned, is far greater than that claimed for it by the makers (1/10 that of adrenaline). In certain invertebrate tissues, too, it has been shown that " epinine " actually elicits a response in greater dilutions than adrenaline (HOGBEN and HOBSON (6)). The effects of " epinine " dilutions of 1 in 100,000 and 1 in 10,000 are shown in fig. 7 : 1 in 10,000,000 and 1 in 1,000,000 had been administered previously : 1 in 2000 is also shown. This last causes a loss of amplitude and then even arrest for a time, to be followed by the " grouped " contractions of LUCIANI. It was noted

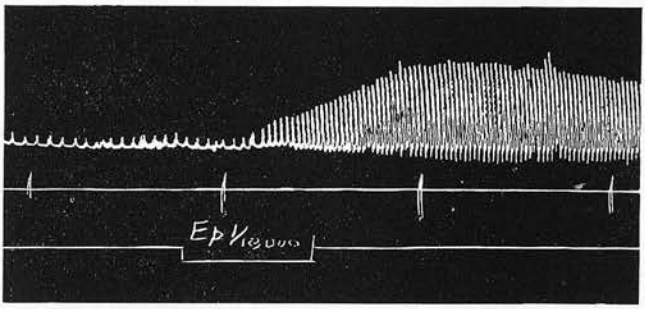

FIG. 8.—Revival of beat on adding epinine, 1/10,000, for 40 seconds.

repeatedly that following adrenaline perfusion the ventricle missed every third, fourth, or tenth beat. BURRIDGE (1) describes the same phenomenon in the frog-heart following perfusion with increase of K.

In fig. 8 is shown the remarkable effect of " epinine " in reviving a heart that is beating slowly and feebly, like the hypodynamic heart of CLARK (4), or the Ca poor heart of BURRIDGE (2). Without postulating with BURRIDGE any " lubricant " action for adrenaline, one may agree with ZUNZ (10), that the heart is more susceptible to drugs when suffering from " perfusion exhaustion " or the like. The frequency here is increased from 19 to 40 per minute, and the amplitude is multiplied sevenfold. This beneficial influence, however, cannot be relied upon, as thereafter beats may be missed or contractions may become grouped. As instances of this, the following may be quoted. Twice a heart was stopped by perfusion with ergotamine tartrate (1 in 10,000) ; partial recovery followed long perfusion. On trying adrenaline now, in the hope of a reversal, a response similar to that of fig. 8 was given, but on returning to stock solution the beats became grouped, a group lasting for about a minute with two-minute intervals.

Certain published tracings of the action of adrenaline on the perfused

frog-heart suggest that the changes therein attributed to adrenaline may be due in whole or in part to changes in the cH of the perfusing fluid. A series of hearts was perfused in which first the effect of a change of cH was registered, then that of adrenaline in the same cH. Solutions were buffered with phosphate and adjusted with HCl or NaOH, using indicators. The " soloid " product at the greater cHs involved adjustment with acid. No effect, even on a perfectly fresh heart, was given by adrenaline in a medium of cH$10^{-8\cdot2}$ or less. pH 7·6 gave a perfectly typical response, and all intermediate values to pH 5·8, the usual lower pH limit for rhythmical contractions. Reference has already been made to the effects of the change from pH 6·6 to 5·9. That for 6·6 to 6·1 is now described.

10.40. Perfusing fluid pH 6·6. Rate 30 per minute.
10.42. Perfusing fluid pH 6·1. No change for 95 seconds. Then markedly slowed for 30 seconds. Recovers with slight gain in tone (not in amplitude or rate).
10.45. Return to pH 6·6. Rate 32 per minute.
10.47. Adrenaline 1 in 100,000 in pH 6·1. Heart stops in extreme relaxation within 30 seconds, the lever falling well below the normal level of diastole. After arrest for 50 seconds, it recommences, quickly regains its pace, gradually recovers its tone, and gains in amplitude by 15 per cent.

Further, if this heart be again perfused with fluid of pH 6·1, or even of pH 5·8, which usually stops the heart rapidly (fig. 2), no apparent change is produced. On trial it is found that the maximum cH for contractions has been increased from $10^{-5\cdot8}$ to $10^{-4\cdot4}$ or thereby. Repeated experiments show that this increased tolerance follows adrenaline perfusion but not perfusion with acid, for a normally perfused heart can be stopped time after time by supplying a Ringer of pH 5·8.

Discussion.

From the foregoing it is evident that adrenaline produces on the perfused fish heart the following effects :—

1. An initial and often very striking inhibition in rate.
2. A subsequent increase in amplitude which may be, but is not invariably, associated with appreciable acceleration.
3. An increased tolerance to hydrogen ions.

Of these facts (1) is specially characteristic of the heart of the fish, and cannot readily be correlated with any known effects of sympathetic stimulation of the heart in other vertebrates. The second falls into line with what is typically found in the mammalian heart and, in the writer's experience, in the heart of the frog. Special attention is due to (3),

since this observation refers to a phenomenon which has not been investigated in other cases, and may assist in throwing light upon the actual mechanism by which adrenaline exerts its influence on the contractile apparatus. Had we more precise knowledge of the mechanism of action of the hydrogen ion one might be tempted to suggest an hypothesis. The difficulty lies in the fact that at present we have no certainty as to how far this ion acts on the surface, how far by penetrating into the cell interior.

It has been shown by CLARK (4) that the increased sensitivity of the hypodynamic heart to cNa+cK is dependent upon a diminution of calcium in the muscle. BURRIDGE (3) states that "the actions of $H^{\cdot}$ are determined by the displacement of other inorganic elements in the tissues, Ca salts being important elements so displaced," arguing from his observations on the frog heart. He goes on to compare the action of adrenaline to that of an increase in KCl, and states that this action is antagonised by Ca. On such a basis it seems impossible to explain the augmentor-accelerator part of the response. MINES has postulated a "polarising" action for $H^{\cdot}$: it has "in a pre-eminent degree the power of reducing or reversing the negative charges on surfaces" (9). This reduction might be expected to reduce the permeability of the cell membrane to kations. Since, experimentally, it has been determined that adrenaline increases the tolerance of the heart to $H^{\cdot}$, it is possible that it does so by increasing this negative charge, thus increasing the permeability to kations. Of those the first to act would be $H^{\cdot}$ and $K^{\cdot}$, from their greater mobility; but to small changes in these the heart quickly adjusts itself, while the effects of changes in Ca content are much more lasting. The barium response in fig. 1 might have been equally well produced by an increase in Ca, and the response is not unlike that often given by adrenaline. Changes in cH, which would hitherto have stopped the heart ("by reducing or reversing the charge"), may no longer, on account of the greater charge, be adequate, nor may inhibition again be provoked by adrenaline, for the cCa (or the negative charge) may be too great to show any slight increase in cH or cK, yet record the amplitude effect of an increase in cCa itself.

SUMMARY.

The response of the heart of Scyllium to adrenaline and related drugs is described and discussed in terms of its permeability to ions in the perfusing solution.

It is a pleasure to express thanks to Dr ALLEN and the staff at Plymouth for their aid in procuring material, as well as to Dr LANCELOT HOGBEN for his suggestions and assistance in the conduct of these experiments.

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[*From the Proceedings of the Physiological Society*, October 16, 1926.]
Journal Physiology, Vol. LXI.

Adrenaline vaso-dilation. By A. D. MACDONALD and W. SCHLAPP.

Small doses of adrenaline (0·002 mg.) may depress the blood-pressure of the anæsthetised cat, and various explanations have been given for this phenomenon. It does not appear to have been realised or appreciated that this fall of pressure is very specially related to the anæsthesia; it is marked under ether and urethane, less marked under chloralose if no ether has been given just before. In the high pressure decerebrate cat from which all traces of ether have been thoroughly ventilated and after the circulation has had time to recover from the effects of anæsthetic, no such depressor response can be evoked, although it was well-marked shortly after decerebration and the level of blood-pressure remains scarcely changed. Depressor doses may now produce prolonged and considerable rises, often interrupted by a notch suggesting a double seat of action of the drug. On ether being supplied to such an animal the depressor response quickly returns.

If, as Burn and Dale(1) suggest, adrenaline vaso-dilation be due to the production in the lungs of minute amounts of a histamine-like substance, that substance can only be produced or liberated under the influence of the anæsthetic, for the blood-pressure response to small doses of histamine itself is little affected by the nature or degree of the anæsthesia in an animal with high blood-pressure.

(1) Burn and Dale. Journ. of Physiol. 61. p. 185. 1926.

THE ACTION OF DRUGS UPON THE MOVEMENTS OF THE STOMACH.

By E. D. M'Crea and A. D. Macdonald. From the Department of Physiology, University of Manchester.

(With six figures in the text.)

(*Received for publication 22nd September* 1928.)

Introduction and Literature.

The results which have been obtained in this laboratory ((12), (13)) from stimulation of the nerves of the stomach with the organ *in situ*, have led us to investigate under similar conditions the action of certain drugs on that organ. We have been especially interested in drugs which appear to stimulate or paralyse autonomic nerves, but have also examined the actions of such general nerve-stimulants as strychnine, and of drugs such as histamine and extracts of the posterior lobe of the pituitary body, which are reputed to act directly on plain muscle.

We have been surprised to find that while many papers and text-books give detailed descriptions of the actions of drugs on the excised stomach or plain muscle preparations, they yield almost no information as to their effects on the organ *in situ*. Yet the clinical and physiological importance of the latter is obvious, for the exsected muscle-slip in oxygenated Ringer-Locke may respond quite differently from the organ as a whole and under more natural conditions.

Previous Observations.

Adrenaline.—The action of adrenaline was investigated in the cat by Elliott (9), who found that the viscus was relaxed; this observation was confirmed by Cannon (3), who stated that tonus is abolished and peristalsis ceases. Carlson, Boyd, and Pearcy (4) observed that adrenaline had both a motor and an inhibitory action on the cardia of the cat and dog, the former when the muscle was hypotonic, the latter when hypertonic; on the stomach as a whole its effect was chiefly inhibitory in both cat and dog. Thomas (17) similarly found in the dog that the hypertonic pylorus was relaxed, the hypotonic contracted, though it is stated by Smith (15) that the sympathetic innervation of the pylorus is excitor.

Pilocarpine and Physostigmine.—Doyon (8) found in the dog that

pilocarpine produces increased contraction with marked rhythmical movements; sometimes, however, before this period of excitation, there is a brief arrest of the movements. BATTELLI, working with the cat, rabbit, and dog, recorded very energetic movements after pilocarpine, but stated that the inhibitory fibres of the vagus are excited, while after physostigmine, which also causes a marked increase in movements, the motor influence of the vagus is augmented.

Atropine.—This drug was observed by CARLSON, BOYD, and PEARCY to abolish both vagus and splanchnic action on the cardia, but to affect the heart more readily than the stomach. BATTELLI also found that the effects of both vagus and of splanchnic stimulation are lost, and that contractility and movements disappear. CUSHNY (6), however, stated that atropine is without effect on vagus and splanchnic control; its effect "is in fact only detected by the cessation of unusual movements induced by certain poisons and by some pathological conditions."

Nicotine.—BATTELLI found that nicotine provokes contractions with small doses, but that after large doses the organ becomes inexcitable. DOYON made similar observations.

Strychnine.—According to BATTELLI this drug is without effect on the stomach, but DOYON observed some increase in tone and movements following its administration, sometimes preceded by a period of arrest.

METHODS.

Our technique has been that employed by M'CREA and M'SWINEY. Briefly, after tying the pylorus, the entogastric pressure is recorded by means of a catheter inserted through the œsophagus and connected to a water-manometer. Saline at 37° C. is conveniently given through a T-piece to distend the stomach. The general condition of the animal is controlled by a simultaneous record of blood-pressure taken from the femoral artery. Such a record also helps to distinguish between a response resulting from a direct effect on the stomach and one due to circulatory disturbance. Between injections of drugs (intravenous in all our experiments) we have interpolated nerve-stimulations for comparison. The experiments have been carried out on thirty cats and six dogs, under ether or chloralose anæsthesia. We have also used decerebrate and spinal cats to eliminate the complicating factor of an anæsthetic.

RESULTS.

Of the sympathomimetic drugs, we have examined most thoroughly the action of *adrenaline.* This almost invariably produces a fall of entogastric pressure, with diminution or cessation of the rhythmical movements; the magnitude and duration of the effect varying according to the dose injected (fig. 1, A). It is interesting to note that the effect

is well marked with doses which depress the blood-pressure. Now stimulation of the splanchnic nerve has been shown to result in either inhibitor or augmentor effects on the stomach (12), according to the "tonus" of that viscus. Only occasionally in the cat have we found an augmentor effect with adrenaline, and then slight and only with large doses. An augmentor result is more easily and more markedly elicited in the dog (fig. 1, B), but never on a scale comparable to the rise

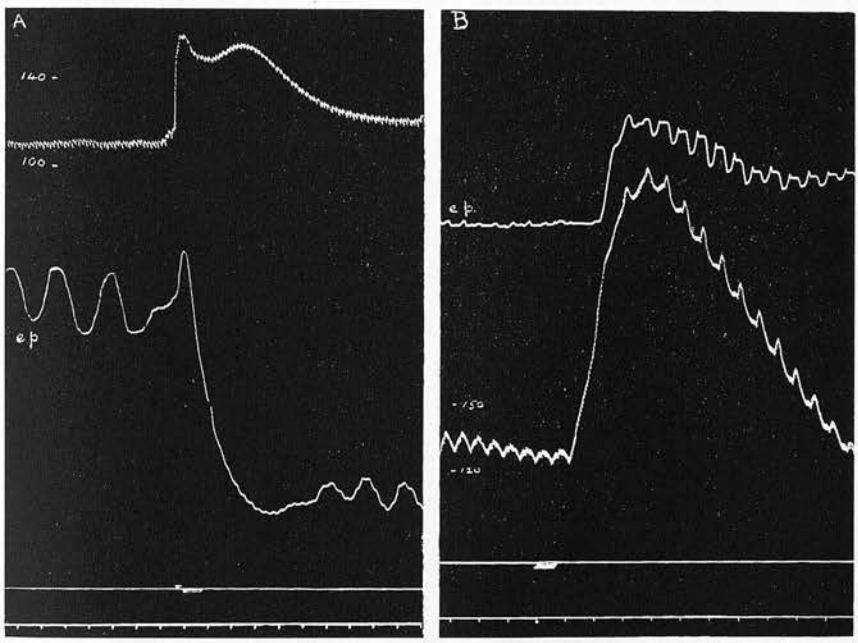

FIG. 1.—Injections of 1 c.c. adrenaline, 1 in 80,000.

A. In cat with high entogastric pressure.
B. In dog with low initial entogastric pressure. *ep*=level of entogastric pressure.
All time-tracings show intervals of 10 seconds. All tracings reproduced are from animals under chloralose anæsthesia.

in entogastric pressure which follows splanchnic stimulation under similar conditions. This augmentor effect with adrenaline is shown in an active organ in which pressure is, for the time being, low. We have seen pyloric movements on these occasions. *Ephedrin* in non-toxic doses acts similarly to adrenaline, but the action on the gastric musculature is not prolonged (as is the action on the circulation). After ephedrin the response to adrenaline, both circulatory and gastric, is markedly increased and prolonged, as if the animal had been sensitised.

Ergot preparations are also held to be sympathetic stimulants. One, therefore, reads with surprise that ergotoxine (10 mg.) "causes marked peristalsis throughout the whole intestinal tract in rabbits" (16). We have used the ergotamine tartrate of the Sandoz Company. This drug

is not well tolerated when given intravenously in cats in doses of more than 2 mg.; doses up to that size are adrenaline-like in their action. Since ergotamine in adequate doses abolishes motor sympathetic effects, we should not expect a reversal of the normal adrenaline inhibition; nor does such reversal occur. But, within a few minutes of administration of ergotamine, we repeatedly observed that the inhibitory action of adrenaline may be diminished or even absent, while the pressor effect on the circulation is enhanced. Thereafter, the stomach resumes its normal response, although the circulatory effect is now depressor.

Nicotine, in sub-lethal intravenous injections, affects the entogastric record in the same manner as a large dose of adrenaline, causing

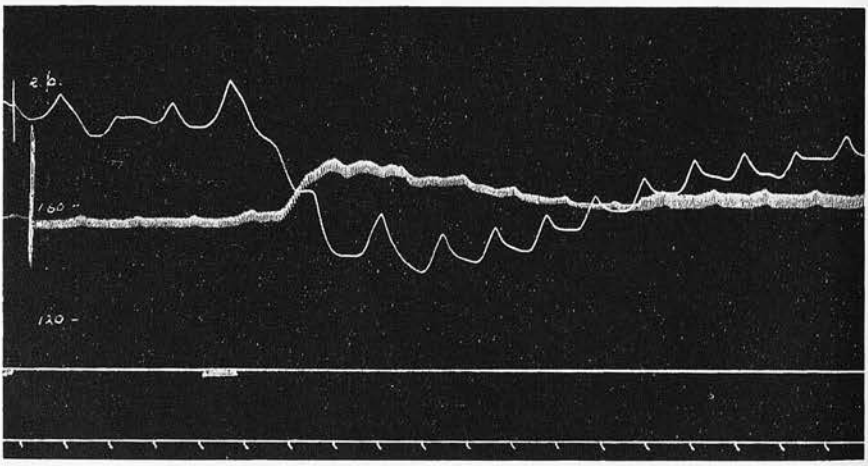

FIG. 2.—Intravenous injection of 0·02 mg. nicotine. (Cat.)

a marked fall in pressure (fig. 2) and often cessation of movements. This effect is probably in part due to the profound vaso-constriction. We have seen nothing of the powerful contractions described by some authors. Such contractions may be due to local irritation, for there is no sign of them with intravenous administration.

Turning to parasympathomimetic drugs, we find in *pilocarpine*, *physostigmine*, and *acetyl-choline*, three, which, when exhibited in suitable doses, are closely similar in their action to the effect of stimulation of the vagus. *Acetyl-choline* provides the closest parallel quantitatively, and its effects are not unduly prolonged, although less marked than with the others. With a high entogastric pressure there is a fall of base-line and movements are diminished or cease; with a low initial pressure a rise is produced and movements are initiated or augmented. Similarly, with *pilocarpine*, doses of 1 mg. can be used to demonstrate both inhibitory and augmentor responses in the same experiment (fig. 3, A and B), according as the entogastric pressure is initially high or low.

Frequently after one or two such doses of pilocarpine, large irregular contractions sweep in succession over the stomach; they persist for two hours or more. These contractions are especially well marked under chloralose anæsthesia or in the decerebrate animal. *Physostigmine* we have found to act in a manner similar to that of pilocarpine, but its effects on the circulation are more protracted and it has to be used in smaller doses because of its greater toxicity.

Atropine, in doses of 0·1 to 0·15 mg. reduces entogastric pressure

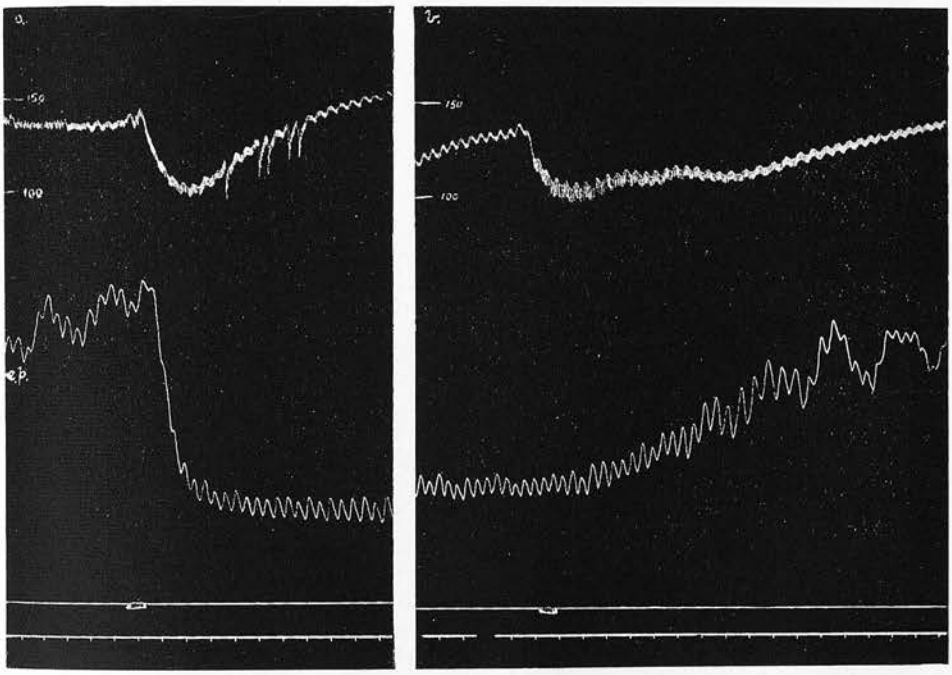

FIG. 3.—Two intravenous injections, each of 1 mg. pilocarpine nitrate, into a cat. There was an interval of 20 minutes between these injections.

and arrests movements of the stomach-musculature for the duration of any one of our experiments, that is for at least four hours. All further responses to drugs or to stimulation of its nerves are abolished (fig. 4), even though the cardio-inhibitory mechanism may not be completely paralysed. This is in complete disagreement with the authorities quoted by CUSHNY (6). We are also unable to confirm the statement (11) that further relaxation can still be produced by stimulation of the vagus. Our technique might not have permitted the recording of this; but it must be insignificant, since we have seen no sign of it either on stimulation of the vagus or with pilocarpine.

Strychnine, in ordinary dosage, has no marked effect on the stomach-pressure or movements, in our hands. With doses sufficient to produce

convulsions, some rise of pressure occurs, but we lay no stress on this observation.

Of drugs believed to act directly on plain muscle, *histamine* in small doses causes a rise of entogastric pressure and augmentation of movement, but the effect is as transitory as it is on the blood-pressure (fig. 5).

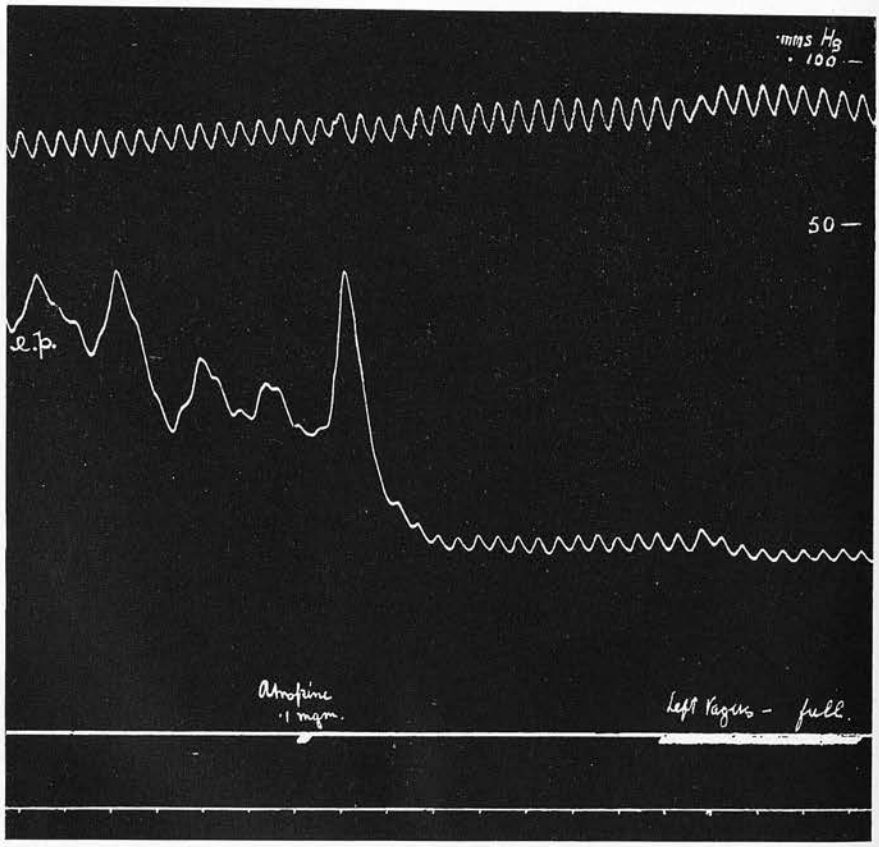

FIG. 4.—Injection of 0·1 mg. atropine and subsequent stimulation of left vagus nerve. (Cat.)

Laboratory extracts of the posterior lobe of the *pituitary body* (histamine-free) cause a fall of entogastric pressure and cessation of movement (fig. 6). GALAN (10) claims that the excised stomach is stimulated by pituitary extracts, but, from the earlier work of one of us (14), we suspect this to be a histamine effect. The effect we record in the intact animal is presumably secondary to the vascular action and similar to that obtained for the intestine by DALE and LAIDLAW. The stomach shows a striking and prolonged blanching as a result of the injection of the pituitary extract—more marked, perhaps, because of the

distension, than in other parts of the gut, and useful for purposes of class demonstration.

Discussion.

A consideration of our results shows that the parallelism between the effects of faradic stimulation of the extrinsic nerves of the stomach and

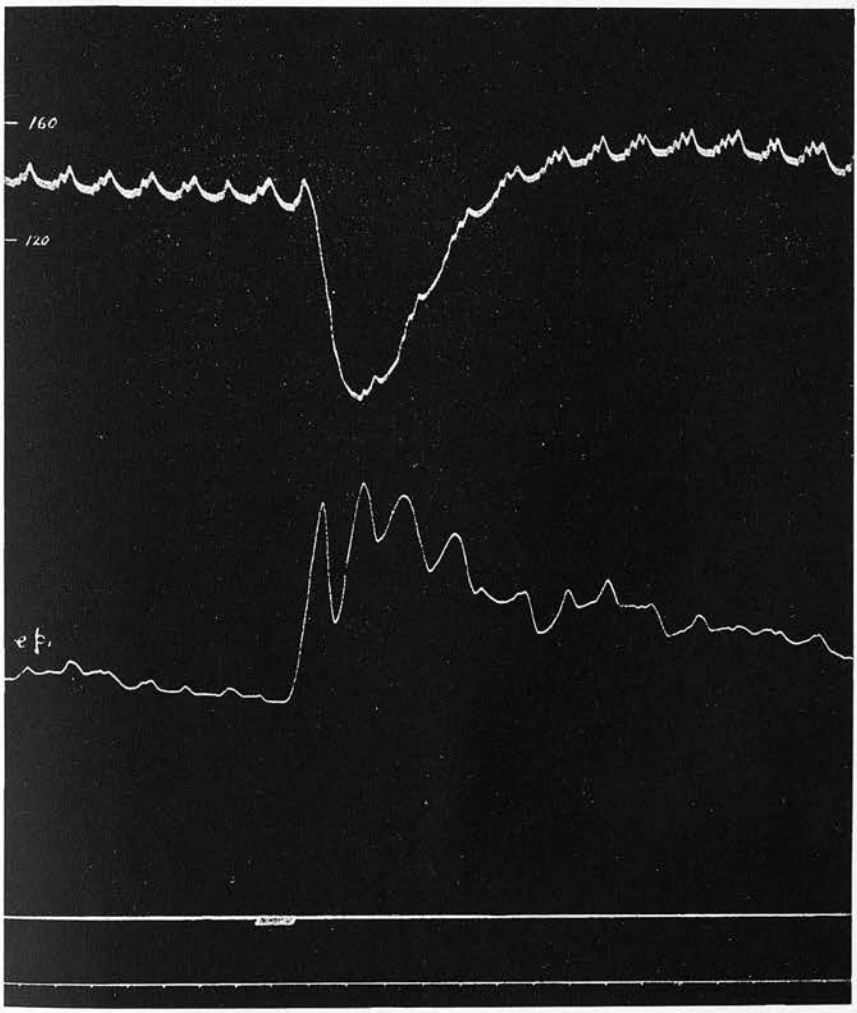

Fig. 5.—Intravenous injection into cat of 0·02 mg. histamine.

those produced by the intravenous injection of sympatho- and para-sympatho-mimetic drugs is less perfect than one had been led to believe. In particular, marked augmentor effects are rarely obtained with adrenaline, yet these are easily elicited on stimulation of the splanchnic nerves. However, apart from the prolongation of their effects, we have abund-

antly satisfied ourselves that pilocarpine and physostigmine can act either as augmentors or depressors of entogastric pressure, just as can

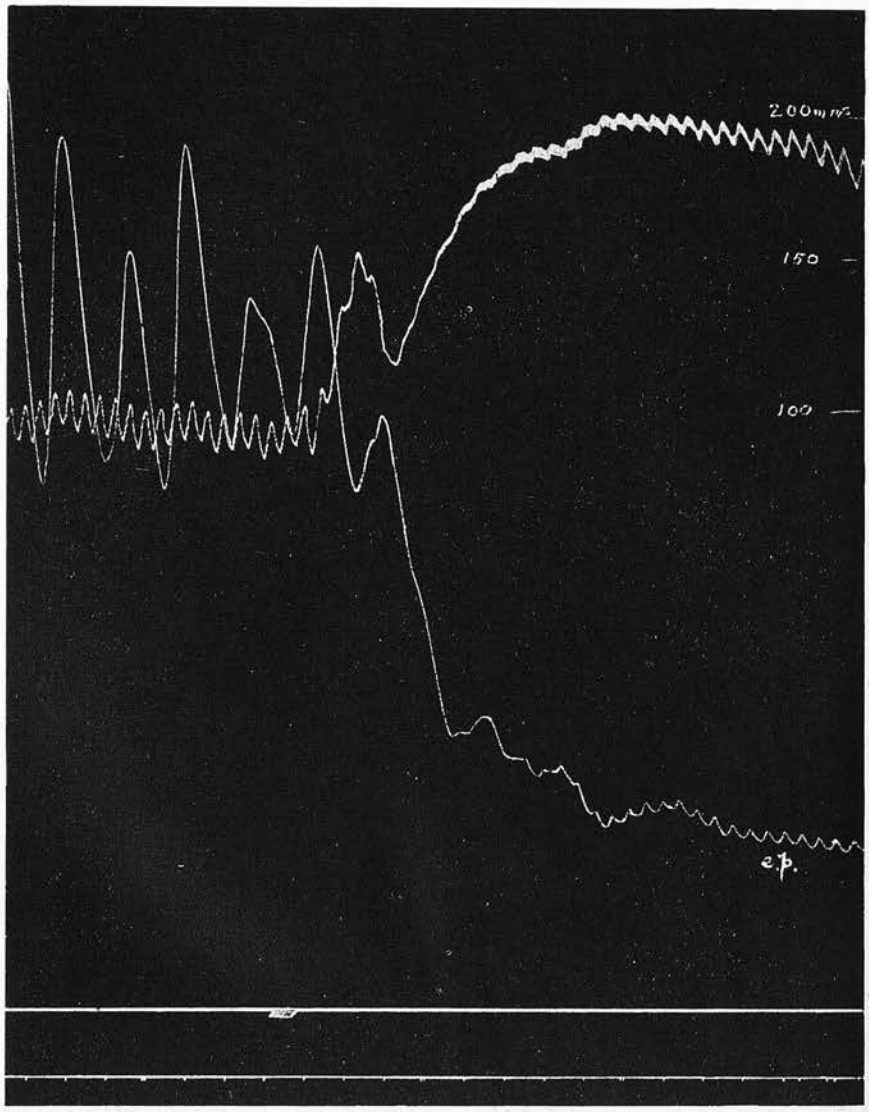

FIG. 6.—Injection of a saline extract of 2 mg. of dried posterior lobe pituitary gland (depressor-free) into cat.

The stomach-movements did not return for over half an hour.

stimulation of the vagus. It is noteworthy, too, that the stomach can show large and irregular contractions long after the circulation has apparently completely recovered from the action of these drugs. And, even if quiescent, very slight stimuli applied to any of its nerves may again pro-

voke wild movements. With acetyl-choline these after-effects are absent, so that this drug more closely imitates the action of the vagus; moreover, we know that acetyl-choline is rapidly destroyed in the tissues (5).

Since the text-books show discrepancies on the point, we would emphasise that atropine, when given even in quite small amounts, completely paralyses the gastric musculature, eliminating both vagus and splanchnic control. After its use, no other drugs that we have tried produce any effect on entogastric pressure.

These experiments with drugs confirm the conclusions drawn from our experimental stimulation of the nerves of the stomach, namely, that the theory of the vagus nerve being excitor and the splanchnic inhibitor is untenable. Both are capable of either an augmentor or inhibitory action upon the stomach, according to its state of "tonus" at the time. We are therefore led to believe that there exists an optimum position of the "postural activity" of the stomach, at which its motor functions are at their maximum. This optimum is not a fixed point, but depends upon such factors as the volume of its contents, the stage of digestion, the state of the circulation and of that of its nervous mechanism. However, at any given moment the viscus may be in a state of equilibrium which is not necessarily the optimum, and then stimulation either through a nerve or by a drug tends to produce an alteration towards the optimum, and results in either increase or decrease of entogastric pressure with alteration of character of movement. The change may overshoot the optimum position, and there then appears a condition in which tonus and movement vary inversely. A rise of tone is ordinarily associated with increased movements, a fall with cessation of movements. But this relation is not invariable; movements and tonus may, under certain conditions, vary inversely instead of directly.

Summary.

1. Adrenaline may produce both inhibitory or augmentor effects on the stomach of the cat and dog, but its action is chiefly inhibitory. It cannot be said to duplicate the effects of faradic stimulation of the splanchnic nerves.

2. Pilocarpine, physostigmine, and acetyl-choline, like the vagus, can be both augmentor and depressor to the stomach, according to its condition of tonus. Pilocarpine and physostigmine often cause irregular after-contractions of considerable duration.

3. Atropine relaxes and paralyses the stomach-musculature and abolishes all response to nervous stimulation or to other drugs.

4. The effects of ephedrine, ergotamine, strychnine, histamine, and pituitary extracts are briefly described.

5. An explanation is advanced of the varying results of nerve-stimulation and of drugs.

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OBSERVATIONS ON THE CONTROL OF THE BLADDER.—THE EFFECTS OF NERVOUS STIMULATION AND OF DRUGS.

By A. D. Macdonald and E. D. M'Crea. From the Department of Pharmacology, University of Manchester.

(With seven figures in the text.)

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CONTENTS.

Introduction.

The observations recorded in this paper were made in order to compare the nervous control of the bladder with that of the stomach, another hollow viscus with comparable innervation which has been extensively investigated in the Manchester laboratories and described in this journal (8, 9). It has been shown that, while the sympathetic nerves to the stomach are commonly regarded as inhibitor and the parasympathetic as excitor, both nerves may, under varying conditions, cause either inhibition or excitation. In general the contracted stomach is relaxed and the relaxed organ is contracted by stimuli, whether nervous—through the vagus or splanchnic nerves—or chemical—by the intravenous injection of certain drugs. The ease and the minimum of operative interference with which the contractions of the bladder can be recorded greatly facilitate such investigation.

The results which have been obtained, whilst presenting certain similarities, are not quite parallel to the observations made on the stomach. They add little to the results of previous workers taken as a whole except on one or two points, but serve to explain some of the bewildering discrepancies which are encountered in the voluminous literature on this subject.[1]

[1] The literature is extensively treated in the review by Fearnsides (5) and in Dennig's monograph (2), and therefore need not be reconsidered here.

Methods.

The method of recording changes in the volume and tension of the vesical contents has been of the simplest nature. A catheter, with lateral and terminal apertures, is passed into the bladder, usually through a supra-pubic opening in the urethra, and is joined by tubing fitted with a T-piece to a water- or mercury-manometer. The T-piece is used to change the contents of the bladder, warm saline being added or withdrawn as required. Supra-pubic exposure of the urethra is a simple operation; we have found no need for the removal of the symphysis pubis as described by Sherrington (10), nor is there any tension on the neck of the bladder. A water-manometer of 20 mm. bore has proved the most useful in these experiments, eliminating the risk of overflow; the delicacy of movement of the piston recorder, bearing a simple frontal writing point, is greatly improved by interposing a layer of liquid paraffin or castor-oil between the water and vulcanite float.

While with this method the volume of the bladder content is permitted to vary, the action of the mercury-manometer, against which all contractions are approximately isometric, is avoided. The pelvic and hypogastric nerves are exposed through inguinal incisions and the whole procedure is extra-peritoneal; transverse division of the recti muscles between the inguinal incisions relieves tension and frees the bladder from gross changes in intra-abdominal pressure. Exposure and handling of the bladder, which result in extreme contraction, have been avoided. When we have wished to observe the contractions directly, the abdomen has been freely opened in the mid-line, the wall caught up at several points to make a bath, which is filled with saline at body temperature. Cats and dogs have been used for these experiments, and in many cases the condition of the animal has been controlled by a record of the blood-pressure. Experiments have been carried out on animals under ether, chloralose, urethane, and luminal anæsthesia, and also on spinal and decerebrate preparations. Experiments in which the catheter has been introduced through the bladder apex and tied in with a purse-string suture have shown no modification of the responses, thus excluding any reflex disturbance caused by the presence of a catheter in the urethra. It will be seen that the vesical responses are profoundly modified by the nature and degree of anæsthesia.

Nervous Control.

Pelvic Nerves.—The parasympathetic control of the urinary bladder is carried out through the pelvic nerves, and most authorities agree—see Dennig (2) and Fearnsides (5)—that in the cat and dog the pelvics are the principal motor nerves. We have noted, as have several workers, that stimulation of the peripheral end of a pelvic nerve in an acute

experiment is mainly unilateral in its effect. Stimulation of an intact nerve results in a strong contraction of the homolateral half and a weaker contraction of the contralateral half of the bladder. We have sometimes observed such contraction to be followed by relaxation (especially if the viscus is active and is maintaining a pressure); this

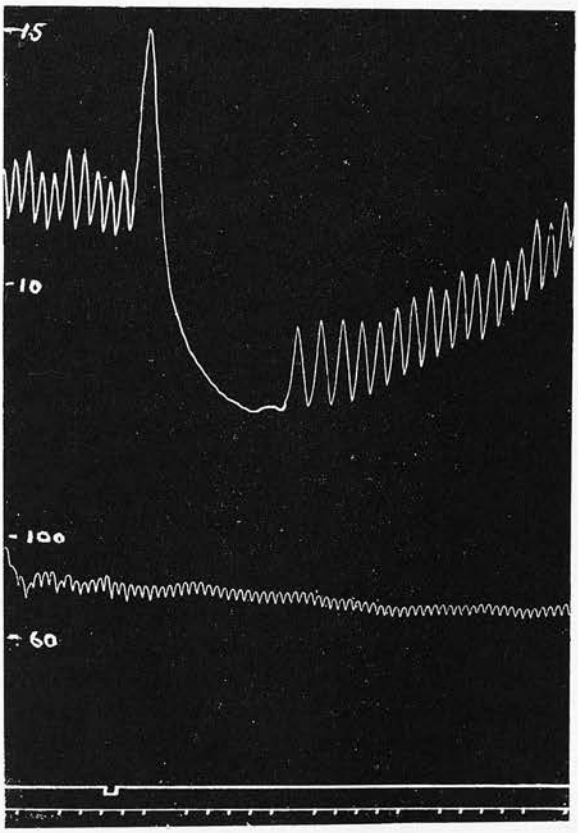


FIG. 1.—Cat, chloralose. All bladder nerves intact. Stimulation of right pelvic nerve. Coil distance, 12 cm. The after-inhibition produced after 5 seconds' stimulation is much the most striking part of the response; in this animal hypogastric stimulation produced only excitation.
Time, 10 sec.
All intravesical pressures in cms./water, all blood-pressures in mm./Hg.

relaxation may even appear during actual stimulation. At times strong and prolonged stimulation may be required in order to produce this relaxation, but in other experiments the loss of tonus and rhythmic movement is easily and rapidly produced. In fig. 1, for example, it appears after 5 seconds' stimulation, and is much the most striking part of the response. Indeed, it resembles a hypogastric rather than a normal pelvic effect. Relaxation, like the contralateral contraction, is a central or reflex action, and can only be obtained by stimulation of

the intact nerve or the central end of the divided pelvic nerve. It resembles the late effect seen by a number of workers on section of the pelvic nerves in recovery experiments, but is here of course of short duration only. Section of the hypogastric nerves does not influence this response; it is thus independent of these nerves. Such relaxation *may* be due to the failure of impulses normally passing *via* the pelvic nerves to reach the bladder and maintain its tonus and rhythm, rather than to the presence of inhibitory fibres in the pelvics. On the other hand, it will be seen, later, that the drugs usually described as parasympathetic can also produce inhibition, and that without any initial contraction.

Hypogastric Nerves.—The sympathetic control of the bladder is considered at length by ELLIOTT (3). He describes three types of control in different animals:

(1) The ferret and, to a less extent, the male goat, in which hypogastric stimulation produces general contraction.

(2) The cat, pig, and monkey, in which there is general relaxation.

(3) The dog, rabbit, and perhaps man, in which there occurs contraction of a narrow area of the base of the bladder, and in which there is no general inhibition comparable to that in the cat.

Earlier workers have recorded widely diverse effects on stimulation of the hypogastric nerves. Thus GRIFFITHS (6), in the dog, describes relaxation in a contracted viscus, contraction in a flaccid organ. COURTADE and GUYON (1), also in the dog, record contraction, LANGLEY and ANDERSON (7) contraction or contraction followed by relaxation, occurring in cats, dogs, and rabbits, and FAGGE (4) notes contraction of the trigonal region of the dog. We believe that our experiments on the cat and dog explain these discrepancies; it has been too generally accepted that the sympathetic nerves of the bladder are purely inhibitor and the parasympathetic excitor. We have found, employing ether as the anæsthetic, that the bladder of the cat is commonly relaxed on hypogastric stimulation. Twice, however, it has been noted that with light anæsthesia the response is motor, later becoming inhibitor as the anæsthesia deepens. When certain other anæsthetics are used motor effects are regularly elicited on sympathetic stimulation. ELLIOTT, using A.C.E. mixture, sometimes failed to obtain the usual hypogastric inhibition in the cat; in these cases he states that he was unable, on dissection, to find vesical branches from these nerves. Under urethane he found the bladder "atonic and useless for the study of inhibition," but we have succeeded in obtaining relaxation by the adjustment of the depth of anæsthesia. It is only atonic with excess of anæsthetic. Under light urethane anæsthesia a motor response can be elicited; under moderate anæsthesia (fig. 2), well-developed inhibition. Precisely similar effects are obtained when sodium luminal is the anæsthetic. We cannot confirm the differences claimed between the effects of hypo-

gastric stimulation in the dog and cat, which have been of degree rather than of kind in the six experiments we have made on the dog, using, when available, females of about 4 kilograms. With ether anæsthesia, hypogastric stimulation gave the inhibition shown in fig. 3, although lighter stimulation produced slight increase in tone. In the chloralosed

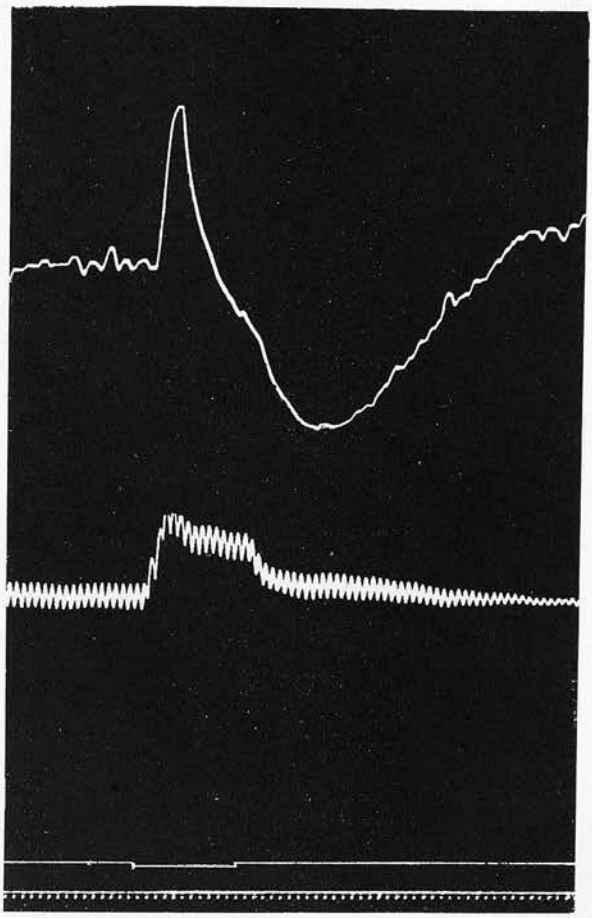


FIG. 2.—Cat, urethane. All bladder nerves intact. Stimulation of right hypogastric nerve. Coil distance, 12 cm. No ether was given to this animal, yet a good inhibitory response is seen.
Time, 5 sec. in this and subsequent tracings.

animal the hypogastrics produce considerable excitation, which is not limited to the trigonal region. When chloralose is given intravenously to an etherised animal and ether administration discontinued, the effect of hypogastric stimulation alters from inhibition to excitation as the ether disappears. These contractions are certainly not confined to the base of the bladder, they are general and well marked (though most evident over the homolateral side) in both cat and dog. As they occur

in bladders showing considerable tonus and rhythmical activity, there is no question of the viscus being "atonic and useless for the study of inhibition"—indeed, on stimulation of the pelvic nerves inhibition may be marked. Conversely, when ether is administered to a chloralosed animal, the motor response to hypogastric stimulation is gradually replaced by the usual inhibition. The contractions evoked by hypogastric stimulation though not so large are often better maintained than those found after pelvic stimulation. The effect of stimulation of the

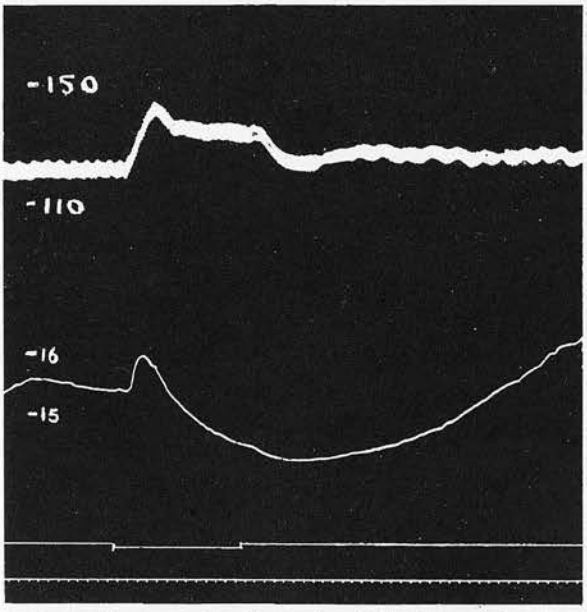


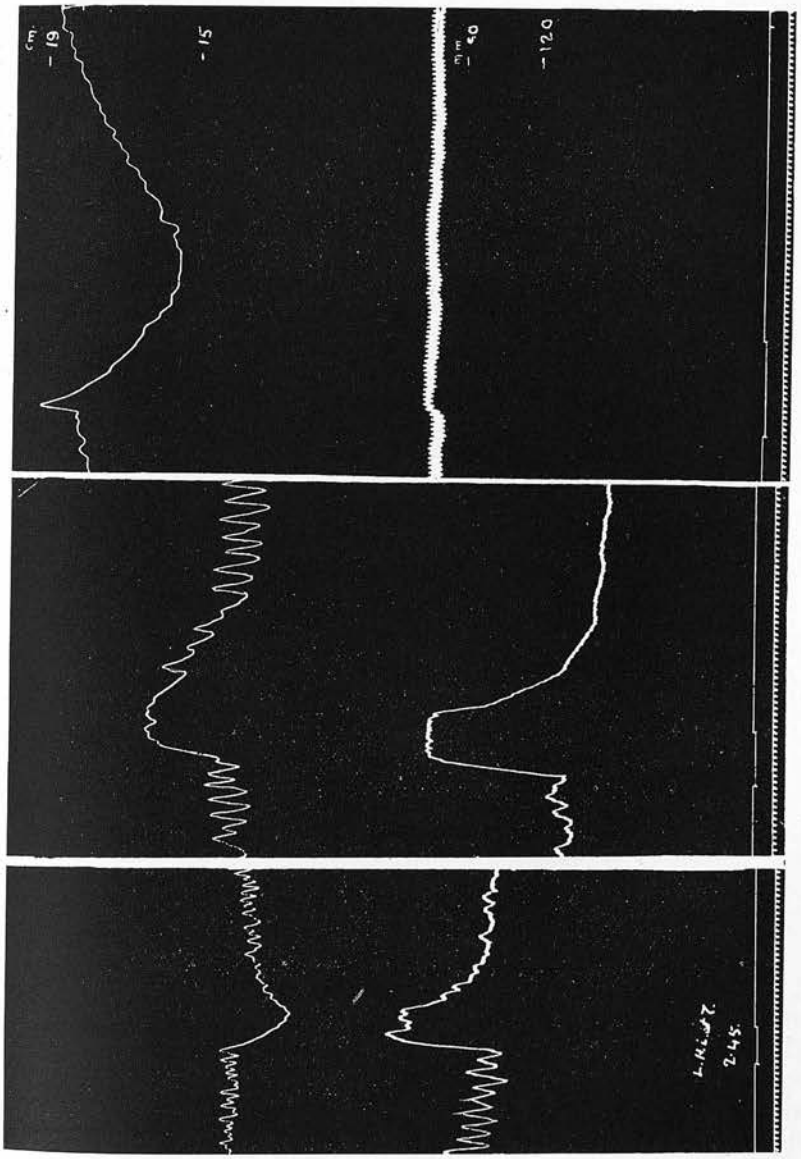
FIG. 3.—Dog, ether. Hypogastric stimulation (coil distance, 5 cm.) gave inhibition. Weaker stimuli gave only excitation.

pelvic nerve resembles an exaggerated rhythmical contraction, and tonus is rarely affected apart from temporary after-inhibition, whereas it is often markedly increased by hypogastric excitation.

We have attempted to eliminate the effects due to anæsthetics by carrying out experiments on decerebrate and spinal preparations. In the decerebrate cat, prepared under ether, we find inhibition at first on hypogastric stimulation. This, however, disappears with the anæsthetic and is replaced by contraction, the inhibition returning if ether is again supplied. In the spinal cat, with the slower loss of the anæsthetic, it has proved more difficult to obtain an active bladder, but in certain instances we were successful, and the hypogastrics proved excitor.

Where a motor response is elicited through the hypogastrics, it can be reversed by the action of ergotamine (fig. 4). It is possible to interpret this in the usual way, namely, that ergotamine paralyses the motor sympathetic. It is noteworthy, however, that ergotamine itself con-

siderably increases the tonus of the bladder, and in fig. 4 (*c*) we might regard the motor sympathetic as fully stimulated, rather than paralysed, and further stimulation of a mixed nerve then produces inhibition.



(*a*) (*b*) (*c*)

FIG. 4.—Cat, chloralose. Three hypogastric stimulations at coil distance 7 cm.
(*a*) While still in some measure etherised: inhibition. (*b*) One hour later, with most of ether ventilated: excitation.
(*c*) Half an hour later, after ergotamine (femergin *it*, 2 c.c.).
Here there is again inhibition, after a slight initial contraction; it will be noted that both vascular and vesical tone have been greatly increased by the ergotamine.

THE ACTION OF DRUGS.

The effects described in the following experiments were obtained by the intravenous injection of the drugs investigated. Of the sympatho-

mimetic drugs, adrenaline, ephedrine, and ergotamine have been examined. Adrenaline, as the result of ELLIOTT'S work, has come to be regarded as duplicating, on the bladder, the effects of hypogastric stimulation. It is certainly possible to get all the effects described for

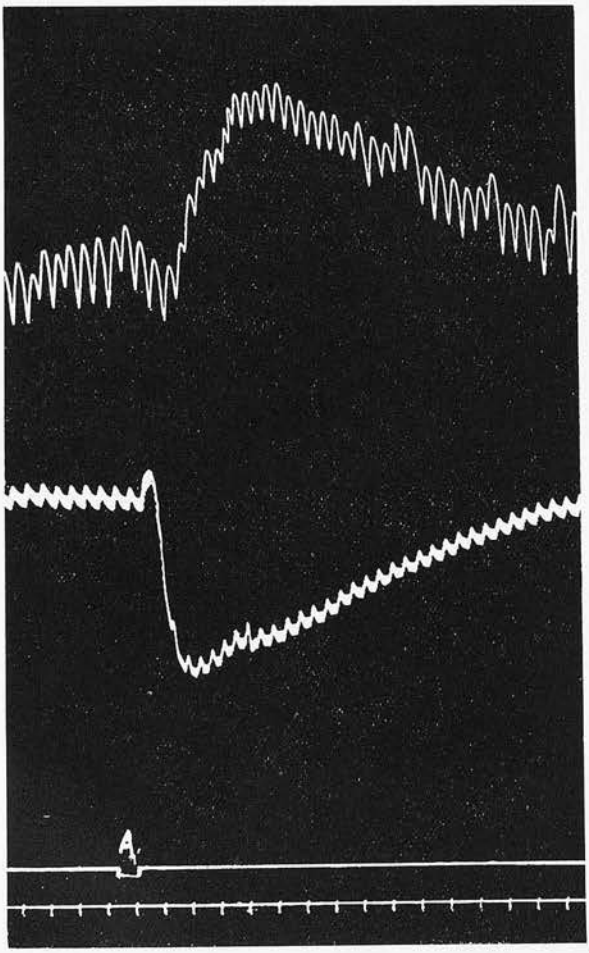


FIG. 5.—Cat, chloralose. At signal, injection of 0·002 mg. adrenaline HCl. Fall in blood-pressure, but considerable increase in tone and rate of bladder movements. In the same animal, when the bladder contents had been so reduced that rhythmical contractions ceased, adrenaline evoked the contractions again.

the hypogastric with adrenaline, but there are certain differences. When adrenaline inhibits the bladder, it usually does so without producing any initial contraction; on the other hand, excitation with adrenaline is commonly a little delayed and may show a preliminary inhibition. The delay is perhaps owing to vaso-constriction impeding the access of the drug to the viscus in adequate concentration. When contraction begins, however, it is usually more marked and prolonged

than that excited by hypogastric stimulation (fig. 5). Extremely small doses of adrenaline are adequate. Even such as 0·002 mg., which produce a fall of blood-pressure in the anæsthetised animal, result at times in a contraction showing an increase of pressure of 8–10 cm. of water. The fact that such contractions are recorded alike with either rise or fall of blood-pressure suggests that circulatory changes are of little significance in the determination of the response.

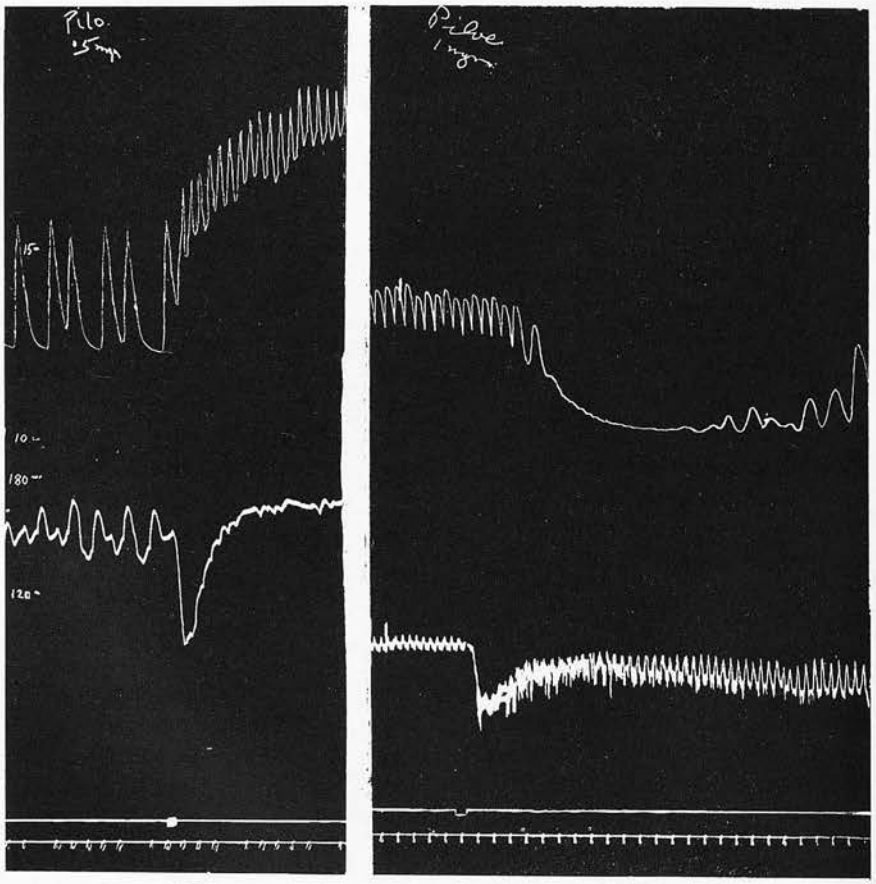
In a bladder in which there are no rhythmical contractions, and especially if ether is the anæsthetic, it is usual for adrenaline to have an inhibitory action. Under light ether anæsthesia, and usually when chloralose is the anæsthetic, a quiescent bladder is stimulated to marked rhythmic contraction with increased tonus, and this persists for a time. Adrenaline can thus initiate contraction as well as augment existing contraction in both size and rate. We find adrenaline much more powerfully excitor to the bladder than to the stomach, and the bladder response is not lost after atropine as is that of the stomach.

Ephedrine, even in considerable dosage (as much as 10 mg. of the hydrochloride intravenously), has little effect on the intravesical pressure; we found tonus and movement to be increased, rather than diminished (as they were by adrenaline in the same experiment).

Ergotamine has been referred to previously; in full doses it causes a marked increase of tonus, reducing the rhythmic movements. The increased tonus is well maintained, and probably favours the production of the relaxation thereafter on hypogastric stimulation or injection of adrenaline.

Of the drugs classed as parasympatho-mimetic, we have chiefly used pilocarpine and atropine. Pilocarpine is usually a powerful excitant, as one would expect, and produces a well-maintained increase in tonus and rate of contractions (fig. 6 (*a*)). At times after pilocarpine, contractions become large, slow, and irregular, and further observations on such a viscus are unprofitable. Exactly the same sequence was common in our observations on the stomach (9). At other times, as in fig. 6 (*b*), pilocarpine produces definite inhibition in place of excitation, and both tonus and movement disappear for a period. The inhibitory response of the bladder after pilocarpine injection lacks the initial contraction obtained on inhibition due to pelvic stimulation, just as adrenaline inhibition lacks that seen with hypogastric inhibition. As is usual, all these effects of pilocarpine are antagonised or abolished by atropine. Atropine has a reputation as a vesical sedative, and in some experiments as little as 0·1 mg. has sufficed to abolish rhythmical activity and markedly reduce tonus. Atropine, however, never seems to destroy irritability and contractility in the bladder as it does in the stomach—apparently all parts of the autonomic system are not equally susceptible to its action. In some of our experiments on the cat as much as 15 mg. have been injected intravenously without abolishing

rhythmic vesical activity. Indeed, in some animals small doses have increased tonus and movements for a time, with some slight and slow loss of tonus later. Although pilocarpine is no longer effective, adrenaline and stimulation of the sympathetic nerves can still evoke an increase of tonus and rhythmic movement, whereas in the stomach, after atropine, no stimulus, either nervous or chemical, elicited any response.

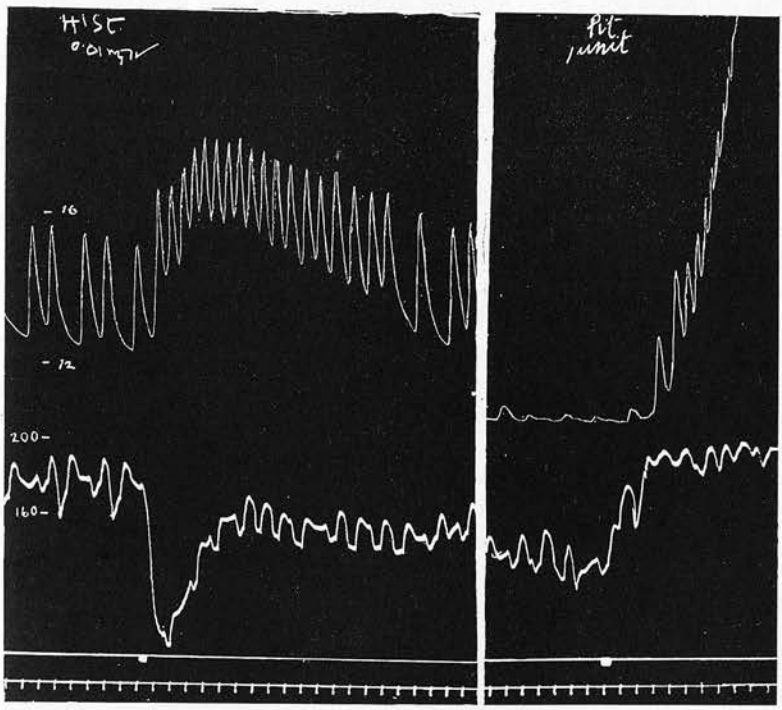


(*a*) (*b*)

FIG. 6.—Cats, chloralose. (*a*) 0·5 mg. pilocarpine, producing increased tone and rapid contractions. (*b*) 1·0 mg. pilocarpine, in a different cat, producing loss of tone and rhythmical movements.

As direct stimulants of plain muscle, we have employed histamine and pituitrin; tracings of the effects of these in the chloralosed cat are shown in fig. 7. Histamine produces an increase of tonus and rhythm lasting for some minutes, even with small doses (fig. 7 (*a*)). The action of pituitary extracts is frequently sudden, and the contractions are enormous and maintained. This occurs even in a previously quiescent viscus.

In the experiment illustrated in fig. 7 (*b*), the intravesical pressure rose from 11 to 28 cm. of water in a minute or two, and only gradually subsided, but rhythmical contractions and some increase of tonus persisted throughout the duration of the experiment. By no other means have we succeeded in producing such impressive effects.



(*a*) (*b*)

FIG. 7.—Cat, chloralose. (*a*) 0·01 mg. histamine, causing a transitory increase in tone and rate of movements. (*b*) 1 unit pituitrin. The intravesical tension rose 7 cm. above the upper edge of the smoked surface, and the total increase was 17 cm. of water. This increase was lost slowly and only partially, and rhythmic contractions persisted throughout the experiment.

DISCUSSION.

In the bladder, as in the stomach, it is clear that the effects of any given stimulation, either nervous or chemical, can be predominantly excitor or inhibitor, according to the existing conditions of the viscus. If the degree of the tonus relative to any given condition of the stomach were known, its behaviour on stimulation could be forecast fairly accurately; with the bladder other factors have to be taken into account, which are comparatively less important when working on entogastric pressure. Firstly, intravesical tension can often be modified within wide limits without reversing the effects of a particular stimulus. Secondly, we have found, in some instances, that changes in the strength

of the stimulus considerably modify the response. Thus, in the etherised dog, strong stimulation of the hypogastric nerve gave the inhibitory response seen in fig. 3, while weaker stimuli were purely excitor. Thirdly, the duration of the stimulus is also an important factor. In certain of our experiments prolonged stimuli were required to elicit pelvic inhibition, in others it appeared at once. The nature and depth of anæsthesia are of paramount importance in all these experiments. With certain anæsthetics—ether, urethane, and sodium luminal—the motor effects of sympathetic excitation are easily lost, especially under deep anæsthesia. These anæsthetics may act by paralysing the motor sympathetic apparatus, or it may be that their own stimulant effect is so marked that further motor response is impossible, though this is unlikely since the administration of ether to an animal otherwise anæsthetised causes a fall of tonus and diminished movement.

In the chloralosed, decerebrate, and spinal animals motor effects are more readily elicited than inhibition.

We believe that our results reconcile the apparently divergent results of other workers, and that, in particular, ELLIOTT'S views require considerable modification since the importance of the anæsthetic influence upon the response to nerve stimulation and the injection of drugs does not appear to have been fully realised. It is also necessary again to call attention to the danger involved in applying to a viscus *in situ*, with intact innervation and circulation, reactions observed with drugs on isolated or perfused organs and tissues.

It would be tempting to try to apply a theory of humoral excitation and inhibition to the similarities and dissimilarities of the responses evoked in the bladder by nervous and chemical stimuli, but for the present such discussion is too speculative to be profitable.

SUMMARY.

The view that the nervous control of the bladder in the cat is compounded of sympathetic inhibition and parasympathetic excitation is inaccurate. Evidence is provided to show that in the cat and the dog the sympathetic and parasympathetic nerves are each both excitor and inhibitor. The effects produced by stimulation of a vesical nerve depend upon at least two factors: muscle tonus and the nature and degree of the anæsthesia. These observations are confirmed and expanded by comparison with the actions of appropriate drugs.

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PRE-SACRAL SYMPATHECTOMY AND THE URINARY BLADDER

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The surgery of the autonomic nervous system is modern and though first extended to the vesical nerves in 1898 by Jaboulay, it is only within the past ten years that the operation with which we are concerned—pre-sacral sympathectomy—has been practised. This operation was introduced by Cotte for the relief of certain gynæcological conditions, but he also applied it to the bladder.

The operation destroys the sympathetic nerve supply of the bladder and it is our intention to show that the indications for, and the results to be expected from, this procedure are not yet defined. It may be said without fear of contradiction that in spite of the attentions of many brilliant workers the functions of the autonomic nervous system are even now only beginning to be understood, and it is certain that our knowledge of the nervous control of the bladder is incomplete. We feel that although the operation of pre-sacral sympathectomy may have a place in the surgery of the bladder it will suffer discredit if too much is expected from it. Furthermore, if it be based upon too dogmatic assumptions, disappointment may prevent the making of valuable clinical observations as to its effects.

Every new venture in surgery is to some extent experimental and we learn both from the failures and the successes. At the same time there must be some basic reasons for attempting any new operation, and we permit ourselves to quote and criticise some of those given for resection of the sympathetic nerve supply of the bladder. First, it has been, and often is, said that the hypogastric nerves are inhibitor to the detrusor muscle and motor to the vesical sphincter. The statement may contain a germ or rather more than a germ of truth, but is most certainly too dogmatic. Stimulation of the peripheral cut end of a hypogastric nerve may produce either inhibitor or excitor effects on the detrusor of the cat and dog (McCrea and Macdonald). The " funnel neck " of the bladder which is seen in tabes, in injury to the cauda equina (Watkins) and after section of the sacral anterior nerve roots (Dennig) (due to relaxation of the internal vesical sphincter) does not appear to bear any relation to the hypogastric nerves. Section of the nerves does not cause relaxation of the internal vesical sphincter either in the cat (Barrington) or in man. Experimental work upon the nerves has produced no evidence of a convincing nature of either an inhibitor or excitor action on the sphincter, and

Denny-Brown and Robertson deny any such effect. Secondly and similarly these nerves have been termed the " nerves of filling," apparently on the assumption that they possess an inhibitor effect on the detrusor as well as being motor to the sphincter. We are convinced that the position is much more complicated and that theses of this nature are misleading and unwarranted. Thirdly, Learmouth presents a much more detailed and definite list of the functions subserved by these nerves. It is stated that the pre-sacral nerve contains fibres which are : (1) Inhibitory to the expulsive muscles of the bladder ; (2) motor to the muscle around the ureterovesical orifice ; (3) motor to the muscle of the trigone ; (4) motor to the internal sphincter ; (5) motor to the smooth muscle of the prostate gland ; (6) motor to the smooth muscle of the seminal vesicles and ejaculatory ducts ; (7) afferent, conveying impressions of distension of the bladder ; (8) afferent, conveying impressions of pain on spasmodic contraction of the bladder, and (9) vaso-constrictor to the vessels of the bladder.

We believe that even such a list of statements errs on the side of excessive simplicity and that, whilst there is some evidence or authority for every item of the list, yet many of these statements require modification. We are concerned with the bladder itself and intend to limit our review to the present-day knowledge of the sympathetic supply of the bladder with the exception that we must mention that V. Zeissl and also Elliott have described a motor action upon the urethra. We purposely avoid entering upon any discussion of the fascinating theories of the act of micturition.

A full and satisfactory review of the work done upon the hypogastric nerves would necessitate a critical account of present-day knowledge of the nervous control of the bladder and, so vast is the literature, this would require the production of a monograph. Fortunately there already exist two masterly reviews of the subject, the first, which is by Fearnsides, covers the ground up to the year 1917 and the second, which is a monograph by Dennig, reaches 1926. Since the latter date a number of observations made on man have added to the knowledge acquired by the experimental physiologist. First, a short account of the effects obtained by stimulation and section of the hypogastric nerves in animals will be given, as it is upon these that our knowledge of physiological function is founded. Secondly, a number of observations made on man will be noted and their bearing upon the experimental results considered.

Stimulation of the Hypogastric Nerves.—The literature on the effects of stimulation of the hypogastric nerves has been quoted previously by us : " The sympathetic control of the bladder is considered at length by Elliott. He describes three types of control in different animals :—

" (1) The ferret and, to a less extent, the male goat, in which hypogastric stimulation produces general contraction.

" (2) The cat, pig, monkey, in which there is general relaxation.

" (3) The dog, rabbit, and perhaps man, in which there occurs contraction of a narrow area of the base of the bladder, and in which there is no general inhibition comparable to that in the cat.

" Earlier workers have recorded widely diverse effects on stimulation of the hypogastric nerves. Thus, Griffiths, in the dog, describes relaxation in a contracted viscus, contraction in a flaccid organ. Courtade and Guyon, also in the dog, record contraction, Langley and Anderson contraction or contraction followed by relaxation, occurring in cats, dogs, and

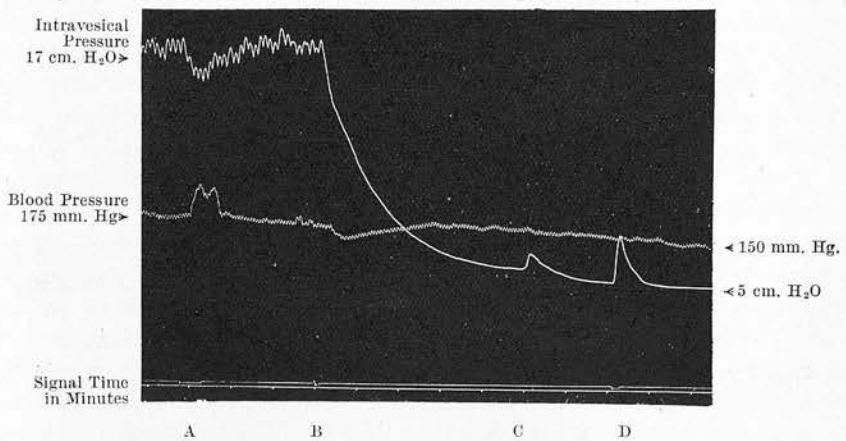

FIG. 1.—Cat, ♂, 2650g. Chloralose. 11/1/33. Record of intravesical pressure (water manometer) and blood-pressure (Hg. manometer). Stimulation of right hypogastric nerve at A and D, before and after a spinal anæsthetic B (20 mg. Procaine HCl). At C, the nerve was pulled on in adjusting the electrodes. " A " is inhibitor, and there is a typical blood-pressure effect. " D " is motor, the intrathecal injection having inhibited tone and movements.

rabbits, and Fagge notes contraction of the trigonal region of the dog. We believe that our experiments on the cat and dog explain these discrepancies ; it has been too generally accepted that the sympathetic nerves of the bladder are purely inhibitor and the parasympathetic excitor."

Actually we have produced evidence that in both animals the effect of stimulation of any nerve trunk on intravesical pressure is conditioned by at least two factors ; muscle tonus and the nature and depth of the anæsthesia. In a viscus which is showing rhythmical variations in pressure and a good level of pressure, stimulation of any one of the four nerve-trunks produces a predominantly inhibitor response. If the pressure be

low, similar stimulation always elicits increased tone and movements. In the case of hypogastric stimulation, this is shown by text-figure 1. A bladder is inhibited by hypogastric stimulation when in tone and showing rhythmical contractions. On decentralising the viscus by giving a spinal anæsthetic—a convenient and cleanly method,—the inhibitor response is replaced by a very definite contraction. Similarly, if stimulation be given at intervals as the effects of the spinal anæsthetic wear off, we find that the motor response gradually develops an inhibitory component, which in time becomes the leading feature of the tracing. Precisely similar results can be obtained by pelvic nerve stimulation, except that the low-pressure contraction is more marked and an initial contraction precedes the relaxation seen at higher pressures (Fig. 2). It is quite

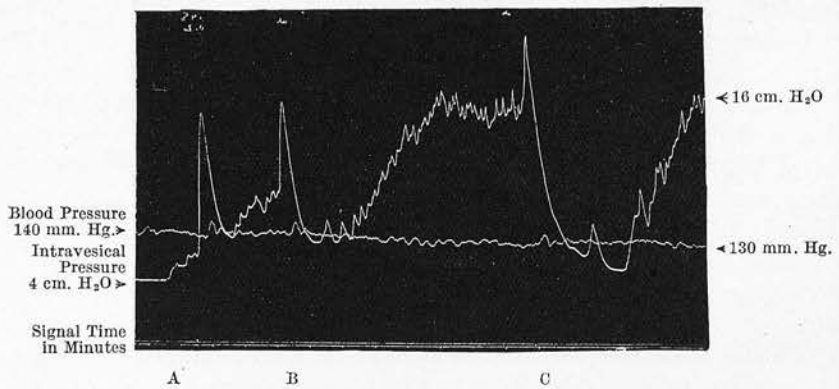

FIG. 2.—Cat, ♂, 3060g. Chloralose, 16/11/32. In a cat in which the hypogastric nerves have been divided, a pelvic nerve is stimulated at intervals. The stimuli are equal in duration and intensity. As the tone of the bladder increases, a purely motor response (A) develops an inhibitory component (B), which eventually predominates (C).

unusual, in our experience, for any hypogastric stimulation to produce as marked an inhibition as that shown in C (Fig. 2).

Barrington's work on micturition may be cited in support of the attribution of both inhibitor and excitor effects to the hypogastric and pelvic nerves. It may be added here that Courtade and Guyon stated that it was the circular muscle coat which responded most markedly to sympathetic stimulation.

The conception of nerve trunks which carry both excitor and inhibitor fibres is, of course, by no means limited to the nerves to the bladder. Indeed, Schafer (1931) has suggested that it is time that physiology should disencumber itself of such merely anatomical terms as sympathetic, parasympathetic, vagotonic and so on, so usual is it for these nerves to

have both motor and inhibitor components. He points out how, in the case of the nerves to blood-vessels, vasodilator fibres were shown to exist in the cervical sympathetic by Dastre and Morat, and in the splanchnics by Kuré, who has also emphasised that the splanchnics contain many motor as well as the more abundant inhibitory fibres to the plain muscle of the alimentary canal. The recent development of the humoral theory of nervous action makes some modification in nomenclature urgent, if we are to think and speak and write at all clearly on autonomic function, and Dale (1933) suggests that autonomic fibres should be classified functionally instead of anatomically, as adrenergic or cholinergic. Recent work has shown that probably all preganglionic fibres are cholinergic, but post-ganglionic fibres do not strictly follow their anatomical classification. Since the response of the detrusor muscle of the bladder to intravenous injections of such drugs as adrenalin, acetyl-choline, pilocarpine and so on varies with the muscle tone, just as it does to nerve stimulation, there is no great gain in exactness in describing the hypogastrics as " predominantly adrenergic but partly cholinergic." It would seem better and simpler to say " mainly inhibitor but also motor (or excitor)."

Section of the Hypogastric Nerves.—The effects of resection of these nerves have been extensively investigated by Barrington and Dennig. Dennig found a normally functioning bladder after section of both hypogastric nerves in the dog, but in cats Barrington observed that slight frequency of micturition resulted. Another observation bearing upon the sensory function of the hypogastric nerves was made by Dennig, who showed that when all nerves with the exception of the hypogastrics were divided, over-distension was still appreciated. This sensation, however, seemed to be less painful than when other nerves were intact. Barrington however, considered that in cats no sensation of distension remained after section of the pelvic nerves. Leriche and Stricker have obtained hyperæmia as an effect of section of the pre-sacral nerve in the bitch, and have caused pain by stimulation of its central cut end.

This brief summary of the results obtained from experimental physiology shows that the hypogastric nerves are not essential to a satisfactory function of the bladder, and that their excitation may, under varying circumstances, produce either contraction or relaxation of the viscus. It would appear that they transmit sensory impulses, particularly of the sense of distension, and, further, that they possess vaso-constrictor fibres. It is not possible to state definitely that any particular area of the bladder always responds in the same manner, although a number of observers have noted contraction limited to the trigonal region. Neither is it possible to attribute a definite function in relation to sphincteric action to these nerves since experimental work has no conclusive evidence

to offer, unless Courtade and Guyon's statement in regard to the circular coat indicates an especial effect on the sphincters, or V. Zeissl's and Elliott's observations on the urethra indicate sphincteric contraction. It would appear wrong to accept the former generalisation of antagonism between the sympathetic and parasympathetic nerve supply of the bladder. It may be noted here that the action of adrenalin does not precisely duplicate the effect of sympathetic stimulation, although often stated to do so. There is certainly no comparison between the relative importance of the pelvic nerves, which are essential to the bladder, and that of the hypogastrics which, although functioning in bladder control, may be dispensed with without marked alteration of function.

The Hypogastric Nerves of Man.—Knowledge gained from observation of the human bladder can now be added to that of the experimental physiologist and comparisons can be drawn. First, Head's work on referred pain may be quoted as in general agreement with the later work of Dennig on dogs. Head showed that the referred pain of retention was diffused over the areas of the 11th and 12th dorsal and 1st lumbar roots, the segmental area of the hypogastric nerves ; whilst that of lesions of the vesical mucosa was referred to the area of distribution of the 3rd and 4th sacral segments, which is that of the pelvic nerves. Macht, working with Wesson and Young, observed that the human trigonal muscle responds to sympathomimetic drugs only and made the inference that it possesses a nervous control different to the remainder of the bladder musculature which was found to respond to both classes of drugs. Young and Macht have based a theory of the mechanism of opening of the vesical sphincter upon this observation. It is an observation paralleled by Fagge and Elliott, who noted in several species of animals a contraction of the trigonal region or muscle on stimulation of the sympathetic. Denny-Brown and Robertson conclude, as the result of an investigation of the bladder in spinal lesions in man, that the hypogastric nerves do not take part in the mechanism of micturition, but that they serve as an inconstant pathway for the pain of powerful vesical contraction, though not for the sensation of normal desire to micturate. A few observations on the effects of stimulation of the pre-sacral nerve have been made and movement of the area about the ureteric orifices and the bladder base have been seen through the cystoscope ; nevertheless, equally experienced workers have failed to observe this, and we ourselves have so far failed in our endeavours in three cases to record vesical pressure change during such stimulation. Learmouth has noted pain on stimulation of the central end of the nerve. Many more observations of the effects of stimulation in man are needed before any definite conclusions can be drawn.

The results of section of these nerves on the normal human bladder

are now quite well known, because the operation of pre-sacral sympathectomy has been fairly extensively practised by gynæcologists (Cotte). The results have not differed from those noted with the animal bladder, and micturition thereafter has seemed to be absolutely normal apart from occasional instances of slightly increased frequency. It may be concluded that these nerves are not essential to a satisfactorily acting bladder.

What are the conclusions which may legitimately be drawn with regard to the function of the hypogastric nerves ? Animal experimental physiology has shown that their stimulation may, under varying circumstances, cause either relaxation or contraction of the detrusor muscle as a whole. The contraction is in some animals perhaps more marked in the trigonal region, perhaps even limited to the trigonal muscle. Evidence as regards sphincteric action is too small to warrant any conclusion, but this lack of evidence is itself against any specific effect on the sphincters. Section in both animals and man may result in slightly increased frequency of micturition or conditions may appear to be unchanged. As regards sensation it seems clear that the distension sense is partly transmitted by the hypogastric nerves and therefore certain sensations of pain. It is probable that the presence of vasoconstrictor fibres can be regarded as established.

Thus far we have endeavoured to show what is known of the action of the sympathetic nerves upon the normal bladder. We would emphasise that as yet there is no proof whatsoever that the hypogastric nerves are either purely excitor or purely inhibitor to the bladder as a whole, and that there is evidence to the contrary. (See Fig. 1 and legend.) Three workers—Fagge, Elliott, and Macht—have obtained excitor effects localised to the trigonal region, but others have failed to note these. We are confident that the mode of action of these nerves is much more complex than it seems is generally suspected, and that, as with the autonomic system in general, much work remains to be done before precise function can be definitely indicated.

The results of section of the pre-sacral nerve for pathological states of the bladder should add in the future to our knowledge of function. A number of observations have already been made by Pieri, Viannay, Cotte, Learmouth, and Foulds. The nerve has been resected in order to relieve the pain of chronic cystitis, tuberculous cystitis, ulceration, and " cystalgia " of doubtful origin. As a result it is claimed that pain can be partly though not completely controlled, and that rebellious inflammations are sometimes cured, perhaps as suggested by Learmouth, because of vaso-dilatation. The operation has been performed in the endeavour to relieve the retention of the " cord bladder " (Learmouth, Foulds), in which it is assumed that the mal-function of the pelvic nerves results in un-

balanced control by the hypogastric nerves. Whatever the soundness of the premisses of this argument, improvement has been noted, and sometimes very marked improvement, but the careful treatment of these special cases and the psychological effects of operation must be duly considered when judging the results. It should be noted that Cotte operated upon several patients with " cystalgia," some of whom had sphincteric spasm but none of the latter were cured, whilst in his other and more successful cases the pain was without spasm. He did not know whether to attribute the beneficial effects to a diminution of tonus in a hypertonic viscus or to some modification of a congested state of the mucosa.

Evidence as to the effects of the operation upon the sexual function is sparse, especially in the male. Cotte states that in the female frigidity does not result: on the contrary in 50 per cent. of his patients it disappeared ; other workers, however, have noted an abnormal frigidity. Cotte observes that impotence did not result in one male upon whom he operated.

Summary

We have shown in our brief review of the subject how many contrary observations have been made. We have shown how a number of these opposite findings have been reconciled as the result of further investigation and in the fullness of time. We hope that in the future carefully controlled observations of the results of pre-sacral sympathectomy will add to our knowledge of the function of these nerves. For the present we know that the sympathetic and parasympathetic nerves function together in the regulation of the bladder and that the parasympathetic are by far the more important. Whilst the action of one or other may be predominantly excitor or inhibitor, yet it is not to be assumed that either is exclusively so, nor yet that they are antagonists. We know that both nerves transmit sensory impulses, the pathway by the pelvic nerves being the more important. We do not know the precise action of the hypogastric nerves on the vesical sphincters ; nor, indeed, do we know that they have any influence at all upon them.

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Pre-sacral sympathectomy and the bladder in man.

By E. D. McCrea and A. D. Macdonald.

Our observations on the effects of nerve stimulation on the bladder of the cat and dog [McCrea and Macdonald, 1930] indicated the presence of both motor and inhibitory fibres in both hypogastric and pelvic nerves, and we concluded that the responses elicited by such stimulations were conditioned by at least two factors—muscle tonus and the nature and depth of the anæsthesia. We have also reviewed the evidence for the commonly held belief that the pre-sacral supply to the bladder in man is inhibitory to the expulsive muscles, apart from the trigone and the uretero-vesical and internal sphincters, and pointed out the serious discrepancies in this literature [McCrea and Macdonald, 1934]. On several occasions we have endeavoured to establish the effects of section and stimulation of the pre-sacral nerve on the tone and movements of the human bladder, but the results in our first three cases were negative as the level and nature of the anæsthesia were such that the viscus was atonic and unresponsive. (These three were gynæcological cases in which pre-sacral sympathectomy was indicated for the relief of various symptoms.)

In a recent male case, operated on for chronic constipation, we were more successful. Anæsthesia after induction was maintained with nitrous oxide and oxygen. After isolation of the nerve plexus the bladder was filled through a catheter and attached to a water manometer. Section of the nerve had no noticeable effect on intravesical pressure, but traction on or electrical stimulation of the peripheral end of the cut nerve resulted in a marked contraction which could be seen to be widespread. Such contractions were observed at varying levels of pressure (30 and 5 cm. of water); they were followed by slight loss of tone, but the motor component was much the more striking part of the response. It is believed therefore that in man, as in the experimental animal, there are motor fibres to the general musculature of the bladder included in the sympathetic supply.

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(22)
see Appendix 6, p. 87.

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Appendix 6, p 87

Report of the Departmental Committee on Dust in Card Rooms in the Cotton Industry

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		3·4	2·6	100[6]	77[6]	
		3·4	2·6	100[7]	77[7]	
		3·3	2·6	100[8]	77[8]	
		3·1	2·2	95[9]	73[9]	
		2·8	1·8	93[10]	67[10]	

APPENDIX VI.

The Presence of Histamine in Cotton Dust, by H. B. Maitland, Harri Heap and A. D. Macdonald, from the Department of Bacteriology and Preventive Medicine and the Department of Pharmacology in the University of Manchester.

The Nature of the Investigation.

The preliminary investigation of Bramwell and Ellis having suggested that card-room workers might be subject to sensitization with cotton dust and that this might possibly be held accountable for some of the disability among them, the problem was taken up with a view to extending the observations. It was desired to establish whether sensitization to cotton dust did in fact exist in cardroom workers and, if it did, to determine what degree of correlation there might be between sensitiveness and disability of an asthmatic type. A method similar to a well known procedure for detecting sensitization to pollen, etc., as a cause of asthma was employed; an extract of the suspected material is made and a small amount injected into the skin of the fore-arm. Sensitization is indicated by the development in a few minutes of a red flush about the area injected, characteristically having a flat raised blanched centre (a wheal). This reaction rapidly disappears and leaves no trace. A subject who is shown by a skin reaction to be sensitive to a particular material develops an asthmatic attack when it is inhaled. In normal persons no skin reaction is caused by the extract.

The first extracts of cotton-dust made by ourselves, when inoculated into the skin of normal individuals, induced a very marked reaction which was found to result from the presence of histamine-like substances, which for brevity we may regard as histamine. This finding seriously complicated the whole problem and widened the field of investigation. It is well known that histamine itself, apart from any process of sensitization, produces, when put into the skin, a reaction indistinguishable from that caused by a substance to which a person is sensitized. It became necessary therefore in using extracts for determining sensitization to ensure that the reaction produced was not due to histamine. This has in fact not yet been accomplished. The scope of the investigation was widened by a consideration of the possible effect that the inhalation of cotton dust might have on the finer air-passages of the lung through the direct action of histamine on these tissues, and apart

altogether from sensitization. Histamine is so potent that very minute amounts might suffice to induce pathological changes.

The work undertaken so far has been preliminary to the study of these larger problems.

The Method used for Detecting Histamine.

Biological methods have been used throughout for the detection and estimation of histamine. These have advantages over chemical methods in being practically specific for histamine, whereas chemical tests are not, and in being about one thousand times more sensitive. Amounts of histamine sufficient to act on tissues fall far below the limits of present-day chemical means of detection.

Extracts in which Histamine was Originally Detected, and their Employment in Tests for Sensitization in Cardroom Workers.

The preparation of these extracts was the work of Dr. Arnold Brown. They were made from very fine dust kindly given to us by Dr. Pickard and represented a certain fine fraction of the dust removed by the Shirley Cage from a mill that was spinning American Cotton.

The extracting fluid was prepared as follows:—

(*a*)	KH_2PO_4	3.68 grammes.
	$Na_2HPO_4.12H_2O$	14.31 ,,
	NaCl	50.00 ,,
	Distilled water	1,000 c.c.
(*b*)	4 per cent. phenol	1,000 c.c.

The two solutions were mixed, and 500 c.c. of the mixture added to 2,000 c.c. of distilled water.

Two samples of dust were utilized.

(1) 25.44 grammes of dust was extracted with 175 c.c. of fluid at 20° C. for 48 hours. It was then filtered through a bacteriological (Seitz) filter.

(2) 15.75 grammes of dust was extracted with 100 c.c. of fluid in a similar manner.

Dr. Brown found that the inoculation of 0.1 c.c. of each extract into the skin of himself and another normal person caused in each instance a very intense reaction. This was so striking that it suggested that histamine might be present, and biological assay indicated that extract (1) contained the equivalent of 0.003 mg. of histamine per c.c. and extract (2) of 0.0015 mg. per c.c.

It was then found that the extracts previously made by Ellis and used by him for testing cardroom workers also contained histamine. This necessitated a reconsideration of the suggestion that the preliminary investigation of Bramwell and Ellis had really made out a *prima facie* case for the existence of sensitization in these men. What they had observed were possibly histamine reactions, a possibility that is strengthened by the fact that only a small number of normal persons were tested as controls and that some of them reacted.

Dr. Brown attempted to separate the effect of histamine from a reaction that would be caused by other substances in the extracts which might be involved in sensitization by trying to find a dilution of the extract at which the histamine effect would be abolished and to use this dilution for testing cardroom workers along with a control solution of histamine containing an amount equivalent to that in the diluted extract. Working on these lines he has tested 56 cardroom operatives but the results were not conclusive as the majority reacted to histamine alone as well as to the dust extract. Before further tests are made it will be necessary to investigate the range of variation in susceptibility to histamine of normal persons; whether there is

anything in the extracts other than histamine that would complicate the result; to determine the most suitable technique for performing the test whether by injection or application to the skin over a small scratch, and to consider the best kind of extract to use.

Attempts to Assess the Value of Various Methods of Preparing Extracts of Dust for Testing Cardroom Workers.

Some experiments have been made to compare the histamine content of extracts of dust made by several methods which have been employed for other materials to which asthmatics are sensitive. It is not known to what extent substances that may be involved in sensitization may be extracted from cotton dust without the simultaneous extraction of histamine.

Two samples of dust from a Shirley Cage were utilized but as it turned out very little histamine was obtained from them, even when the best method for recovering histamine (Soxhlet extraction with alcohol) was used. The conclusion to be drawn from this, in the light of the experiment described in the next section in which fractions of dust were tested, is that histamine may be found in the finer particles of dust and not detected in the whole sample. It is the fine particles too which *a priori* should be regarded as most important from the standpoint of sensitization as they are most likely to reach the lungs.

The following experiments were made with two samples of dust from a Shirley Cage, one (No. 10) from a mill that was spinning American cotton, the other (No. 11), a mixture of American and Brazilian. Neither sample was large enough to permit of fractionation. Each was tested as follows:—

25 grammes were used in each instance and the extraction was carried out at room temperature.

(1) extracted with 100 c.c. phosphate solution for 24 hours.
(2) extracted with 100 c.c. phosphate solution for 6 days.
(3) extracted with 100 c.c. tartaric solution for 24 hours.
(4) extracted with 100 c.c. tartaric solution for 6 days.
(5) extracted with 100 c.c. of 0.75 per cent. NaCl containing 4 per cent. phenol, for 2 days.
(6) extracted with 100 c.c. absolute alcohol for 24 hours, and the residue, after evaporation, dissolved in 100 c.c. of phosphate solution.

The formula of the phosphate solution has already been given. The tartaric solution consisted of: tartaric acid 5 grammes, phenol 40 grammes, water 1,000 c.c.

Each of the twelve extracts was tested biologically and contained less than 0.001 mg. of histamine per c.c.

Sample number 10 (20 grammes) was submitted to Soxhlet extraction with ether for 7 hours and the residue further extracted by Soxhlet with absolute alcohol for 20 hours. Each extract was evaporated and the residue taken up as far as possible in water, heated over a water bath for half an hour and filtered. Each extract represented 20 mg. of residue per c.c. These extracts on biological assay could be assumed to contain less than 0.0001 mg. of histamine per c.c.

Some of each of the samples of dust (Nos. 10 and 11) were sent to Professor Storm van Leeuwen of Leiden, who kindly made extracts and tested them on asthmatic patients as well as on normal controls. He made the extracts by mixing 3 to 5 grammes of dust with 100 c.c. of saline (0.85 per cent. NaCl). This was put in a shaking machine for 2 hours at room temperature; the mass was then filtered through paper and a bacterial filter and 0.5 per cent. phenol added. Both extracts produced reactions in nearly all asthmatics who were tested (presumably not cardroom workers) and failed to give a histamine reaction in the skin of normal persons. That his extracts did not contain histamine was confirmed by

us; he kindly sent us the extracts which were tested biologically. He noted, however, that they caused a late reaction in normals as well as asthmatics which he attributed to some material other than histamine or substances involved in sensitization. These results confirm our own in so far as histamine was not found. The observation that something was present which caused a late reaction is of interest as the reactions produced by Dr. Brown's original extracts were noted to persist for a longer time than the histamine reactions. The fact that van Leeuwen's extracts induced reactions in nearly all asthmatics is more difficult to interpret. It is somewhat difficult to see why asthmatics who may be sensitive to house dust etc. should react to cotton dust. There may be a common factor in various kinds of dust to which persons may become sensitive. The application of this hypothesis to the disability of cardroom workers would however require much further work to confirm or refute it.

Attempts to Determine the Fraction of Dust which contained Histamine.

The distribution of histamine in dust in relation to the size of particle is of interest because only the finest particles are likely to reach the lungs. The original samples in which histamine was found were composed of very fine particles and represented only a small fraction of the dust collected by the Shirley Cage.

A large amount of dust from American Cotton was separated into five fractions, graded according to size of particles. Fraction number four it is believed approximated to the fine dust originally sent by Dr. Pickard. The whole dust and each fraction was extracted with saline solution (0.85 per cent. NaCl) for 48 hours at room temperature. Of each fraction 25 grammes was taken; fractions No. 1 and 2 were mixed with 200 c.c. of saline, fraction 3 with 150 c.c., and fractions 4 and 5 with 100 c.c. When tested biologically fraction number four was the only one that contained histamine.

The whole dust was also extracted with saline containing 2 per cent. phenol; 1,000 g. of dust to 4 litres in one instance and 1,000 g. to 1 litre of solution in another. In neither of these was histamine detected. Totani's reaction was performed on the more concentrated of these two extracts. This is a chemical test for bases containing the glyoxaline ring; it includes histamine but is not specific for it. The reaction was positive, but, in view of the more sensitive biological test for histamine being negative, it can only be regarded as indicating that other bases were present.

Some of the whole dust was extracted with ether and dried, and afterwards extracted with ethyl alcohol. This extract was evaporated to dryness and the residue dissolved in phosphate solution. Histamine was not demonstrated.

It thus appears that histamine may not be distributed equally throughout dust and may be confined to the fine particles. These may form so small a fraction of the total dust by weight that extraction of the whole dust or of other fractions may fail to reveal the presence of histamine in it.

The Effect of Bacterial Action in Producing Histamine during the Process of Extraction.

Van Leeuwen states that extraction (with saline) should last for only one or two hours and be made at a temperature not higher than about 20° C., otherwise histamine might be formed during the process. That cotton dust does contain bases which might be the precursors of histamine is indicated by the positive result obtained with Totani's test. It is known that some bacteria can form histamine when growing in the presence of some of these bases, particularly histidine. It is therefore desirable to avoid this complication by including some bacteriostatic agent, such as phenol, in the extracting fluid when the period of extraction is long enough to permit bacterial growth. It will be noted that this was done in preparing the original extracts, and those made previously by Ellis. Phenol was omitted

from the last experiment in which various fractions of dust were extracted with saline for 48 hours. In these bacteria had grown but there is no certainty that the histamine found in fraction number four was produced by them. Histamine was recovered from a similar fraction of dust in the earlier experiments when the extracting fluid contained phenol.

Summary.

Histamine has been found in the dust collected by the Shirley Cage from cardrooms. Biological methods have been used throughout for the detection of histamine. The present-day chemical means of detection are less specific, about one thousand times less sensitive, and would fail to reveal amounts of histamine sufficient to act on tissues.

It is suggested that probably histamine is associated chiefly with the very fine particles of dust, which are those most likely to reach the lungs by inhalation. The relation of this finding to disability of an asthmatic nature in cardroom workers is discussed. The presence of histamine in cotton-dust has complicated the problem of determining whether such disability is caused by sensitization to substances in dust other than histamine. It has not yet been established that sensitization to cotton-dust does occur.

The possibility that inhalation of cotton-dust might affect the finer air-passages of the lungs through the direct action of histamine on those tissues has to be considered. Histamine is so potent that very minute amounts might suffice to induce pathological changes.

APPENDIX VII.

Preliminary Investigation of Possible Sources of Histamine.

From the Shirley Institute of the Cotton Industry Research Association.

Detection of histamine and allied bases.

The most delicate chemical test for histamine is one which is characteristic of nearly all bases containing the glyoxaline nucleus—the production of a deep red colour with sodium diazobenzene-p-sulphonate in aqueous sodium carbonate (Pauly 2. Physiol. Chem., 1904, **42**, 508; Totani, Biochem. J. 1915, **9**, 385). This colour is given by all glyoxalines which contain a free imino-group and also a hydrogen atom or some other displaceable group, such as a carboxyl group, in one of the 2, 4 or 5 positions. An exceptional behaviour is shown when a carboxyalkyl, carboxyanilide or nitro group is present in the 4 or 5 position, no colour being obtained (Fargher and Pyman, J. Chem. Soc., 1919, **115**, 217, 1015). With histamine, a distinct rose-pink colour is still obtainable at a dilution of 1 in 10,000.

The reaction is not, however, confined solely to bases which contain a glyoxaline nucleus; a very similar colour is, for example, given by tyrosine, so that whilst a negative reaction with sodium diazobenzene-p-sulphonate shows definitely that histamine is either not present or is present in very minute amount indeed, a positive reaction indicates only that histamine or bases allied to it *may* be present. (The depth of colour under standard conditions of testing, however, affords a qualitative comparison of different samples.) On this account, whenever the material available has been sufficient for the purpose, the presence of bases in the purine, histidine and arginine groups has been tested for by precipitation of the silver salts.

It cannot be too strongly emphasized that the identification of histamine in the very minute amounts which may be present in many of the samples examined can be accomplished satisfactorily only by the biological method of testing the direct stimulant effect on plain muscle.

Preparation of extracts

In comparing samples of cotton and the dusts from cotton and other fibres, the following method of extraction and isolation of bases has been employed:—

100 grams of the material were extracted successively with three quantities of one litre of water, two of the extractions being carried out at 40° C. and the third at 90-95° C. The extracts were united, concentrated under diminished pressure to a low bulk, and purified by precipitation with 50 per cent. alcohol, lead acetate and basic lead acetate. After removal of excess lead, the volume was adjusted to 70 c.c. and the bases precipitated with phosphotungstic acid in 5 per cent. sulphuric acid solution. The bases were recovered in the ordinary way, and dissolved in 10 c.c. of water, so that 1 c.c. contained the bases corresponding to 10 grams of the original material. The depth of colour given with sodium diazobenzene-p-sulphonate was determined on 1 c.c. and the behaviour towards silver salts on the remainder.

Materials examined.

In the preliminary tests, the following materials have been examined:—

(*a*) Three " normal " cottons, an American Texas, an Egyptian Sakel, and an Indian, Broach.

(*b*) An Egyptian Sakel cotton infected with " black leaf."

(*c*) An American cotton (probably African-American) badly attacked by stainer bug, which was forwarded to the Institute as causing considerable inconvenience to operatives not only during carding and spinning but also during weaving.

(*d*) Dusts from coconut fibre and Algerian fibre, neither of which was considered to give rise to the irritating symptoms characteristic of cotton dust. The former contained 4,000,000 moulds and bacteria per gram, moulds being three times as numerous as bacteria; *Mucor* and *Penicillium* were the most common types. The latter contained 1,000,000 organisms per gram; they appeared to be common types—soil bacteria and species of *Mucor* and *Penicillium*. (The figures are very much lower than for cotton dusts, where the number of organisms per gram is of the order 300,000,000).

(*e*) The fine cotton dust in which histamine was detected by biological methods.

The results are summarized in the following Table:—

Sample.	Phosphotungstates (grams, from 100 grams material).	Reaction with sodium diazobenzene-p-sulphonate.	Silver fractionation; precipitate in fraction.			Nitrogen per cent.	
			Purine.	Histidine.	Arginine.	Total.	Insoluble.
Cotton dust ...	2·6	Faint	Very slight	Very slight	None	—	—
Coconut fibre dust	nil	None	—	—	—	0·72	0·68
Algerian fibre dust.	1·2	Very faint	—	—	—	1·44	0·89
Texan 149 ...	2·5	Definite	Slight	Slight	None	0·18	0·12
Sakel 148 ...	2·3	Definite	Slight	Very slight	None	0·23	0·17
Broach 141 ...	3·4	Very definite	Definite	Definite	Very slight	0·23	0·14
Sakel, black leaf	2·5	Faint	—	—	—	—	—
American stainer bug.	4·1	Very definite	Slight	Slight	None	0·40	0·27

THE EXAMINATION OF COTTON, COIR AND ESPARTO-GRASS DUST FOR HISTAMINE

BY

A. D. MACDONALD AND H. B. MAITLAND

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THE EXAMINATION OF COTTON, COIR AND ESPARTO-GRASS DUST FOR HISTAMINE

By A. D. MACDONALD and H. B. MAITLAND

From the Department of Pharmacology and the Department of Bacteriology and Preventive Medicine in the University of Manchester

(With 1 Figure in the Text)

As a result of some preliminary investigations upon the aetiology of an asthmatic type of disability among workers in carding-rooms of cotton mills, Maitland, Heap and Macdonald (1932) showed that it was possible to extract from some samples of cotton dust a substance which gave the biological reactions of histamine. The dust was that to which affected workers would be exposed. The amount of histamine recovered varied from 0·02 mg. to less than 0·004 mg. per g. of dust. Different bulk samples of dust contained unequal amounts, and the amount also varied in different fractions of one sample graded according to the size of dust particles. It was thought that the finer fractions contained the larger amounts of histamine and that some bulk samples were, relatively at any rate, histamine free.

Two samples of dust (Nos. 17 and 18) have since been examined; they contained histamine in possibly significant quantities. Dust No. 17 came from the Shirley cage in a mill spinning a mixture of American (Texas) and Argentine cotton; sample No. 18 was extracted by a Shirley cage from a mill also spinning American and Argentine cotton and was the very fine dust that had escaped through the gauze of the dust filter.

These samples were fractionated according to the size of dust particles by standard 50, 100 and 200-mesh sieves of the Institution of Mining and Metallurgy. In addition for sample No. 17 a 300-mesh sieve was used; the dust of sample No. 18 which passed the 200-mesh sieve was further graded by the method of air flotation. The sieve apertures were: 50 mesh, 0·254 mm.; 100 mesh, 0·127 mm.; 200 mesh, 0·063 mm.; 300 mesh, 0·049 mm. The following fractions were obtained:

Sample No. 17

17 *a*, too coarse to pass through a 50-mesh sieve.
17 *b*, passed a 50 but not a 100-mesh sieve.
17 *c*, passed a 100 but not a 200-mesh sieve.
17 *d*, passed a 200 but not a 300-mesh sieve.
17 *e*, passed a 300-mesh sieve.

The cotton fibres from the first three samples were removed as far as possible by air blowing. These fractions had the following moisture and ash content:

Fraction	Moisture content %	Ash content (original sample) %	Ash content (dried sample) %
17 *a*	12·22	18·70	21·3
17 *b*	6·04	56·6	60·2
17 *c*	2·89	79·3	81·7
17 *d*	3·36	75·2	78·0
17 *e*	4·16	70·7	73·8

Sample No. 18

18 *a*, too coarse to pass a 50-mesh sieve.

18 *b*, passed a 50 but not a 100-mesh sieve.

18 *c*, passed a 100 but not a 200-mesh sieve.

18 *d*, 18 *e*, 18 *f*, 18 *g*, fractions of the dust that passed a 200-mesh sieve, in decreasing order of size of particles.

To obtain the last four grades, the finest dust contained in the fraction that passed the 200-mesh sieve was separated by air flotation, the particles removed being caught either in settling towers or in an electrical precipitator; the residue was the fraction 18 *d*. The part that was removed was then refractionated similarly, the residue from it being the sample 18 *e*, that caught in the settling towers being 18 *f*, and the very finest fraction caught by the electrical precipitator being 18 *g*.

The size distribution of particles, apart from fragments of cotton fibre, was as follows:

Mean diameter of particles in μ	80	40	32	24	20	16	12·8	9·6	6·4	3·2	1·6	—
% of particles in 18 *d*	3·5	28	31	12	7·5	5	5	5	2	—	—	—
% of particles in 18 *e*	—	1	8	14	21	24	17	9	5	—	—	—
Mean diameter of particles in μ	>10	10	5	4	3	2·5	2	1·6	1·2	0·8	0·4	0·2
% of particles in 18 *f*	3	3	4	7	6	5	6	9	12	33	11	—
% of particles in 18 *g*	—	1	1	2	3	3	3	8	12	25	42	

The distribution of fragments of cotton fibres in the last four grades was as follows:

Fraction	% of total number of particles	Size range in μ (length)
18 *d*	30	100–20
18 *e*	22	80–20
18 *f*	10	40–4
18 *g*	7	20–4

The moisture and ash content of the fractions of sample No. 18 was as follows:

Fraction	Moisture content %	Ash content (original sample) %	Ash content (dried sample) %
18 *a*	13·19	12·2	14·08
18 *b*	7·86	47·3	51·3
18 *c*	2·52	85·0	87·1
18 *d*	1·60	91·0	92·5
18 *e*	2·85	79·6	82·0
18 *f*	5·98	46·6	49·6
18 *g*	7·54	49·1	53·1

The various fractions of dust were prepared for assay as follows:

(*a*) By Soxhlet extraction with ether for about 7 hours. This removed most of the fat and should not have removed histamine, but occasionally a depressor reaction was given by the ether extract—probably because of traces of moisture.

(*b*) By further Soxhlet extraction with absolute alcohol for 24 hours or longer. The alcoholic extract was evaporated on a water bath until it achieved a constant weight. A watery extract of the residue was prepared so that each c.c. was equivalent to 20 or 50 mg. of residue. This solution was tested as soon as possible after it was prepared, and compared with histamine (the ergamine acid phosphate of Messrs Burroughs, Wellcome and Co.).

(*c*) By Soxhlet extraction with water after the ether and alcohol extractions. This usually yielded a coloured but inactive solution.

The alcoholic extract, like histamine, had the following properties. It was a stimulant, in great dilution, to the isolated guinea-pig uterus suspended in a bath of oxygenated Ringer solution. It depressed the blood pressure of the etherised or otherwise deeply anaesthetised cat. It depressed and then raised the blood pressure in the spinal or lightly chloralosed cat. As histamine rapidly loses its activity in dilute neutral or alkaline solution, the solutions were prepared in weak acid on any occasion on which it was inconvenient to assay the depressor substances forthwith, and these solutions were neutralised just before injection. (This depressor-pressor response is due to the combined effects of capillary dilatation and arteriole constriction, the latter being caused primarily by an action on plain muscle and secondarily by stimulation of the secretion of adrenaline. Histamine stimulates most plain muscles and many secreting glands.) The blood pressure effects persisted even when enough atropine had been administered to paralyse the actions of the vagus nerve on the heart, and hence were not due to the choline group. Sometimes other effects characteristic of histamine were noted such as stimulation of intestinal or respiratory movements.

The alcoholic extracts, when compared with histamine solutions of known strengths (Fig. 1), contained as much as 0·01 mg. histamine per c.c., corresponding to a concentration in the dust of 0·01 mg. per g., if one assumes that all the histamine was extracted and that all was preformed. In some extractions about one-tenth of this amount was found. It was found for sample No. 17 that the finest fraction was richest in histamine, but all the fractions contained some. Thus 17 *e* contained about twice as much as 17 *c* and 17 *c* about one and one-half times as much as 17 *a*. The finest fraction of sample No. 18 contained less histamine than the finest (but not similar) fractions of sample No. 17. Thus 18 *g* contained about 0·0004 mg. per g. of dust and 18 *f* about 0·0002 mg. per g.

We have examined in all only a limited number of samples of cotton dust but we believe that histamine is a fairly constant constituent of the dust that is extracted from carding-rooms of cotton mills. It is to be expected that, as we have found, the histamine content of various samples will differ. Our ex-

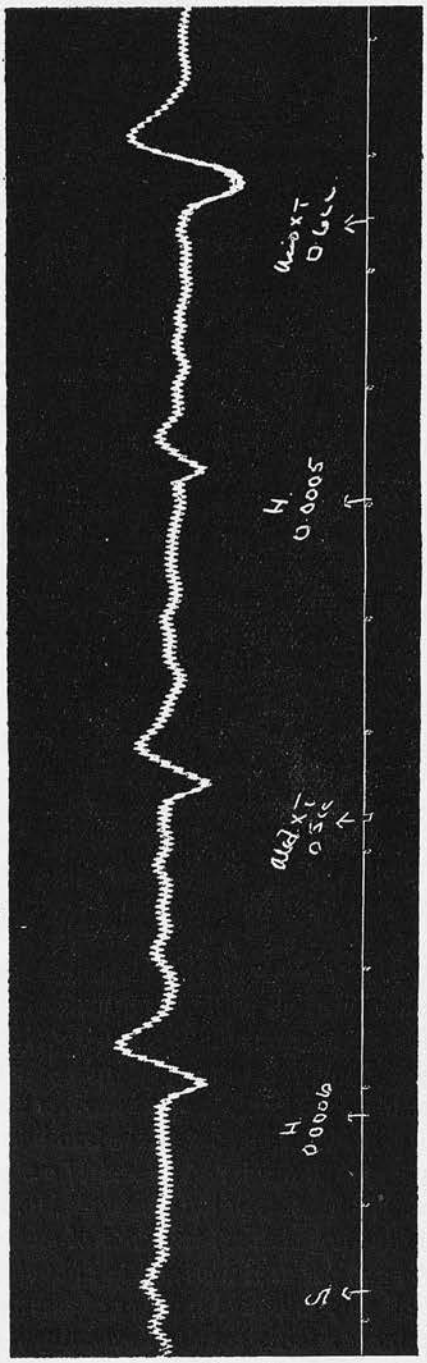

Fig. 1. Cat, ♂, decapitate. 23.iii.1933. Tracing of effects on blood pressure of alternate injections of acid extract of dust, neutralized just before injection, and histamine. *S* is a control injection of saline. Time in minutes. 1 c.c. of acid extract is to be regarded as the equivalent of 1 g. of dust (fraction 17*c*) It will be seen that 0·6 c.c. extract > 0·0006 mg. histamine > 0·5 c.c. extract. 1 g. of dust presumably contains, therefore, about 0·001 mg. histamine. (Tracing reduced by ⅓.)

perience so far also indicates, as we suggested previously, that the finer fractions contain larger amounts than the coarser fractions.

We have had the opportunity to examine one sample of dust from coir (Algerian cocoa-nut fibre) and one sample of dust from esparto-grass. The handling of the esparto-grass produced considerable amounts of a fine black dust. This grass was used for stuffing upholstery. Each sample of dust was extracted and tested as has been described. The alcoholic extracts contained only traces of depressor substances, much less than was found in cotton; esparto-grass dust contained even less than coir dust.

CONCLUSION

The possible importance of histamine in cotton dust as a factor in the causation of the respiratory disability from which card-room operatives suffer has not yet been assessed. Histamine does appear however to be present in most samples of dust to which they are exposed and especially in the finer particles which would most readily reach the lungs. It is of some interest and perhaps suggestive that workers exposed to coir dust and esparto-grass dust, both very dusty occupations, do not suffer from the respiratory trouble found among cotton operatives, and that the single samples of these dusts that have been examined were practically free from histamine.

We are indebted to Mr H. L. Green and Mr T. C. Nugent of the Experimental Station, Porton, Wilts., for fractionating the samples of dust and for supplying the chemical and physical data relating to them; also to Dr W. D. Hood for obtaining samples of coir and esparto-grass dust.

REFERENCE

MAITLAND, H. B., HEAP, H. and MACDONALD, A. D. (1932). *Report of the Departmental Committee on Dust in Card Rooms in the Cotton Industry*, Appendix VI. London: H.M. Stationery Office.

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CONTENTS

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A. D. Macdonald. (24).

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Investigations on Respiratory Dust Disease in Operatives in the Cotton Industry

by

C. PRAUSNITZ

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The detailed chemical separation and purification of the various fractions described is a very difficult problem, requiring highly expert knowledge of organic chemistry which the writer does not possess. Mr. Ernest Haworth has been awarded the Dr. Angus Smith Scholarship of this university for the purpose of carrying on this study.

D. THE PREPARATION OF SPECIMENS FOR BIOLOGICAL TESTS

For biological tests a stock of several grammes of the dried protein has been prepared. In order to obtain sterile material from the very badly contaminated cotton dust the attempt was at first made to filter the protein solutions through Berkefeld or Seitz filters. Unfortunately, the dried protein, when emulsified with normal saline solution in an agate mortar, dissolves incompletely, and the resulting suspension forms a thick, viscid layer on the surface of the filter, thus causing a great quantitative and probably qualitative loss. The method ultimately adopted was the following : The dust was freed from its waxy constituents with petroleum ether ; alcoholic extraction was dispensed with so as not unduly to denaturate the protein. Thorough extraction was then performed with Coca's solution, the liquid was centrifuged, and filtered, successively, through paper, a Seitz " clearing " disk, and a Seitz bacterial filter disk. It was thus possible to remove the ultra-fine dust particles present in the suspension so far as not to interfere with the fine filtration through the last membrane. The whole process of graded filtration was accomplished within a day, the last filtration lasted only a few hours ; hence there was little danger of the loss of active material by adsorption. The subsequent precipitation with ammonium sulphate and dialysis were carried out under sterile precautions, the salt being sterilized in the hot-air oven.

In view of the presence of considerable amounts of histamine in the raw cotton dust it was necessary to ensure its absence in the protein. It was found by biological tests that 1 per cent. solutions of the various protein fractions were free from histamine. Actually, the solutions employed in allergy tests (page 47) were far weaker—1 : 10,000 to 1 : 100,000, and the reactions produced by them have an entirely different appearance from that of histamine reactions (page 47).

PART II.—BIOLOGICAL INVESTIGATIONS

A. ANIMAL EXPERIMENTS

1. The Histamine Problem

By
A. D. Macdonald and C. Prausnitz.

The presence of histamine-like substances in cotton dust was first demonstrated by the investigations of Maitland, Heap and Macdonald (1932). The amount of active substance was seen to vary with different bulk samples and with different fractions of one sample which had been graded according to the size of the dust particles.

A further investigation was carried out by Macdonald and Maitland (1934) with two samples of Shirley cage dust from a Texas and Argentine mixing. The mean amount of histamine estimated in each of them was 0·02 to 0·04 mg. per 100 g. of dust, and in each sample the finer dust fractions were proportionately richer in it than the coarser ones. They also examined a sample of coir (Algerian coconut fibre) dust and esparto-grass dust ; the coir dust gave only a weak reaction, the esparto-grass an even weaker one.

The method employed in their investigations was Soxhlet extraction, first with ether, then with absolute alcohol, and lastly water. Previously only the alcoholic extract had given the histamine reactions, but in the second series they obtained a slight reaction with the ether extract. In the present investigation the Soxhlet method was not used as it was desired to examine larger samples and to obtain, as it were, a cross-section of the whole bulk of the dust. Wherever possible, the sample was passed through the shaker sieve, chief attention being given to the fine dust which was extracted in the stirring machine. Where it was not possible to liberate sufficient dust by shaking the sample, owing to its thickly matted, felted character, 50 g. of the whole material were placed in one-litre gas jars, soaked with the extracting liquid and repeatedly stirred. After two preliminary extractions with petroleum ether (B.P. 40–60° C.) the material was dried and twice extracted with acidulated alcohol ; the total time of alcohol extraction was 2–3 days. The filtered alcoholic extract was distilled down to a small volume at 40–50° C. under a reduced pressure ; the residue in the distillation flask was then evaporated almost to dryness on the water bath, stirred with 10 c.c. of distilled water, and filtered. The liquid was kept in the refrigerator until the time of testing—normally 2 or 3 days, but even after several weeks' storage no loss of activity was demonstrable. Immediately before injection into the animal the material was neutralized.

The routine biological tests consisted in the intravenous injection to cats completely anaesthetized with chloralose. If a typical depressor

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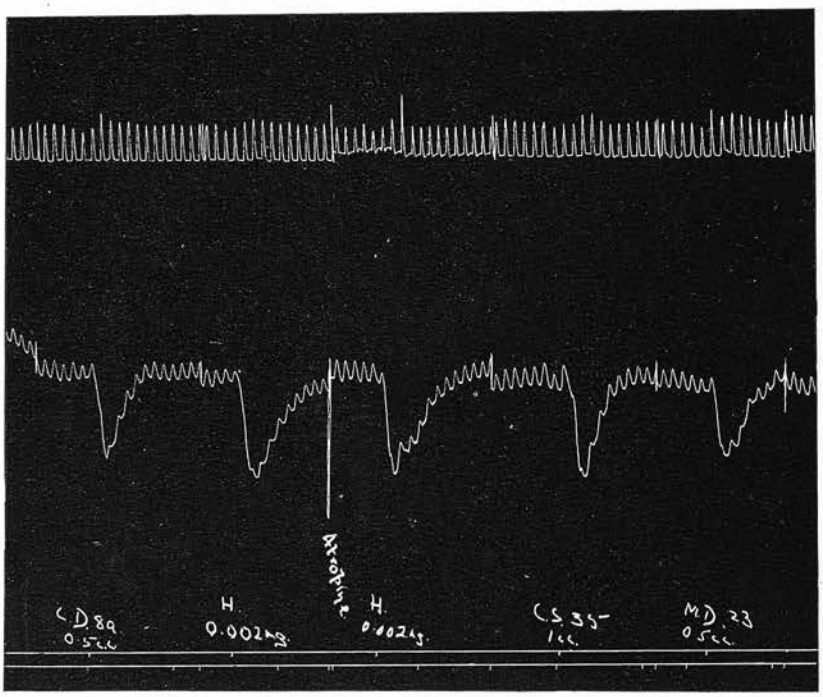

FIG. 5.—Blood pressure curve in chloralosed male cat, weight 3kg. 17.5.34.

The reactions, read from left to right, were the result of intravenous injections of the following substances: Alcoholic extract of cotton dust; 0·002 mg. histamine; 0·002 mg. histamine; alcoholic extract of delinted Russian cotton seed; alcoholic extract of barley dust.

effect was found, the tests were repeated after injection of 1 mg. atropine. As the intensity of the reaction tends to vary with individual cats, control injections were performed in each instance with graded amounts of pure histamine (ergamine acid phosphate of Messrs. Burroughs, Wellcome and Co.). A specimen curve obtained in this manner is shown in Fig. 5. The animals were killed immediately after each experiment.

In one series the results of the intravenous injection were controlled on a strip of cat's intestine suspended in Ringer's solution to which the various test substances were added. The results obtained with the two methods were in close agreement.

The results of the tests performed with cotton dust samples are given in the following table.

TABLE V

Histamine Content of Cotton Dust

N. American, .	Opener	0·006 mg. per 100 g.
,, ..	Cardroom, taker-in ..	0·012 ,,
,, ..	Under carding machine ..	0·006 ,,
,, ..	Cardstripping waste ..	0·012 ,,
Egyptian, ..	Opener	0·008 ,,
,, ..	Scutcher	0·012 ,,
,, ..	Cardroom, taker-in ..	0·012 ,,
W. Indian, ..	Opener + Scutcher ..	0·0015 ,,
N. American +	Argentine, Shirley Cage ..	0·006 ,,

Although certain quantitative differences were therefore observed, there appears to be no doubt that a substance of histamine-like character is regularly present in cotton dust.

The next question was concerned with its origin. As microscopical examination shows the dust to be derived mainly from the seed husks and fibres, these were obtained from a number of different specimens of cotton seed and examined separately. The specimens were kindly supplied by Mr. J. W. Pearson, Managing Director, The British Oil and Cake Mills, Ltd., London. In one case the husks and kernels were examined individually. The seeds were split longitudinally and the kernels extracted with the point of a needle. In some cases the " linters ", i.e., the raw fibres as they had been removed from the seed in the ginning process, were also examined. The kernels were ground in a seed mill and stirred in the machine, the husks and linters were soaked with the extracting liquid in gas jars. The results are summarized in Table VI.

The results of these tests appear to prove conclusively that the histamine-like substance present in cotton dust is already present in the original cotton seed, both the husks and kernels. The small amount found in the linters may possibly be accounted for by assuming them to have been contaminated in the ginning process with fragments of husks. Actually, of course, the linters examined are nothing but samples taken from the raw cotton bales and therefore still containing the cotton dust. By contrast, no histamine

reaction was obtained with extracts of the cardroom " sliver ", i.e., the cotton which had undergone its final purification by removal of all the dust in the carding process, preparatory to its subjection to spinning.

TABLE VI

Histamine Content of Cotton Seed

Egyptian, black, delinted	0·003 mg. per 100 g.
,, ,, kernels	0·0025 ,,
,, ,, husks	0·0015 ,,
East African, delinted seed	0·01 ,,
,, linters	0·02 ,,
West African, delinted seed	0·005 ,,
,, linters	0·005 ,,
Uganda, delinted seed	0·006 ,,
,, linters	trace
Indian, delinted seed	0·012 ,,
,, linters	0·005 ,,
Russian, delinted seed	0·003 ,,
,, linters	0·003 ,,
Cardroom sliver	nil.

At an earlier stage of this investigation, before the presence of histamine in the original cotton seed was known, the working hypothesis was also considered that histamine might be formed in the cotton dust by the action of moulds during transit. Cotton always contains considerable numbers of mould spores, and cotton bales are always found to have an appreciable content of moisture. This is partly due to the hygroscopic nature of cotton fibre and dust. In addition, however, the cotton is often artificially moistened after ginning and before it is pressed. During transit from the ginnery to the port of shipment a little moisture may be lost, and this is often made up for by further humidification. On board ship no loss of water is likely to occur.

The amount of water allowed in Egyptian cotton by the importers is 8·5 per cent.; higher humidity occurs not infrequently, any excess being calculated on the price of the cotton. The average moisture content of bales arriving in this country may be taken to be between 8 and 9 per cent. It has been shown by Balls that at the periphery of the bale the humidity changes from day to day with that of the surrounding atmosphere; it may vary between 2 and 20 per cent. But the central portions of the bale undergo only slow, long-period changes.

Considering the facts that the bales are always more or less moist, that there are plenty of mould spores present, and that there is a large amount of organic dust open to attack by them, the questions had to be studied (1) whether the moulds found in cotton dust could grow under such conditions, and (2) whether they were able to produce substances of a histamine-like character.

From the stock sample of cotton dust several species of moulds present in fairly large numbers were isolated. They were, in the order of their frequency, a *Mucor*, a black *Aspergillus*, a bluish-grey *Aspergillus*, two species of *Penicillium* and a further species, No. 7, which was not identified. All the cultures grew luxuriantly on malt-agar. As there was reason to believe that fairly anaerobic conditions exist in the interior portions of the bales, the possibility of their development under such conditions was tested. Malt-agar plates, inoculated with the various strains, were placed in the anaerobic jar, which was evacuated and filled with mixtures of hydrogen and air at various percentages ; the bottom of the jar was filled with a little water to give sufficient moisture for the development of the moulds. In a mixture of 1 part of air and 9 parts of hydrogen *Mucor* and mould Nr. 7 grew poorly, the others not at all. In a mixture of 1 part of air and 7 parts of hydrogen *Mucor*, mould No. 7 and one *Penicillium* strain showed a moderate, the other strains rather poor growth.

In a further set of experiments half-ounce ointment tins were filled to the brim with fine cotton dust, sterilized in the hot-air oven for 2 hours at 140° C., and dried in the desiccator. Sterile normal saline solution was added to the dust in each tin in varying amounts, so as to make up a moisture content of 5, 10, 15, and 20 per cent. respectively. The moist surfaces of the dust were inoculated respectively with pure culture material of the different strains of moulds ; the tins were hermetically sealed with their covers and strips of adhesive plaster. After 9 days' incubation at 37° C. the 20 per cent. tins showed a luxuriant growth of *Mucor* and both species of *Aspergillus*, whilst the other moulds had only grown poorly. The 15 per cent. tins showed a scanty growth of the black *Aspergillus*, none with the other strains. The tins containing smaller amounts of water gave no growth. Subsequent incubation at room temperature gave the same results.

Although, therefore, the possibility of the development of moulds in the interior of the bales was hardly probable, the question as to their power of producing histamine was investigated. One-litre flasks containing 400 c.c. of Czapek-Dox's medium (Glucose 50, $NaNO_3$ 2, KH_2PO_4 1, KCl 0·5, $MgSO_4.7H_2O$ 0·5, $FeSO_4.7H_2O$ 0·01, H_2O 1,000) and 5g. finely sieved cotton dust were autoclaved and inoculated, respectively, with the different mould cultures. All but mould No. 7 grew very well, the latter moderately. After 10 days' incubation the fluid contents of each flask were drawn off, slightly acidified to prevent decomposition of any histamine present, most of the water was distilled off in a vacuum, and further treatment carried out as described previously for the residue of the alcoholic extracts. A control flask of uninoculated Czapek-Dox's medium was treated in like manner. In every case the histamine reaction was negative. It appeared, therefore, that even the slight amounts of histamine present in the added dust had either been broken down by the moulds or, more probably, destroyed by the autoclaving.

The result of this series of experiments appears finally to disprove the possibility that moulds commonly present in cotton dust have any connexion with its histamine content.

In continuation of the earlier investigations by Macdonald and Maitland fresh samples of coir dust and esparto-grass dust were obtained and treated in the same way as cotton dust. Coir dust contained about 0·0015 mg. per 100 g. dust, whilst esparto-grass dust contained as much as 0·005 mg.

Since respiratory affections somewhat resembling those seen in cotton operatives had been observed rather frequently in maltsters, Dr. J. C. Bridge, H.M. Senior Medical Inspector of Factories, suggested examination of malt dust for histamine. Dr. A. N. Currie, H.M. Medical Inspector of Factories, Glasgow, kindly supplied various samples of such dust. The samples were examined in the manner previously described; the results are given in the following table.

TABLE VII

Histamine Content of Barley and Malt Dust

Whole Barley, Czechoslovakian ..	0·004 mg. per 100 g.
do. Californian	nil.
do. Scottish	trace.
Barley dust from screens	0·002–0·008 mg. per 100 g.
Malt dust from screens (2 factories) ..	nil.

These results prove that a histamine-like substance is present in barley dust at similar orders of magnitude as in cotton dust. Its absence in malt dust is explained by the conditions of heat and moisture under which malt is produced from barley; these conditions acting in a slightly alkaline medium would suffice to destroy histamine effectually.

As a control to the examination of various vegetable dusts, Dr. S. A. Henry, H.M. Medical Inspector of Factories, suggested extending the investigation to wool dust. Through the courtesy of the Manager of Messrs. Paton and Baldwins, Ltd., Wyvern Mills, Melton Mowbray, two samples of wool dust were obtained and examined in the routine manner. Microscopically, the fibres present in each sample were pure animal hair, no trace of cotton fibre was found. The following results were obtained.

TABLE VIII

Histamine Content of Wool Dust

Wool dust, fettling waste	= or < 0·001 mg. per 100 g.
Wool dust, opening machine	= or < 0·002 ,,

Conclusions.—It has thus been possible to demonstrate a substance or substances showing the characteristic biological reaction of histamine in various vegetable and certain animal dusts. Whether

the active substance actually is histamine has not as yet been determined. Very large quantities of dust have to be extracted in order to obtain sufficient material for chemical analysis, and this has not, so far, been possible. With regard to cotton dust experiments in this direction are being carried out with the assistance of Mr. Ernest Haworth (*see* page 25).

The question as to the causal relations between histamine and the respiratory disease of cotton operatives will be discussed in a later section (*see* page 54).

2. Inhalation Experiments with Whole Cotton Dust

The object of this series of experiments was to reproduce in the animal the effects caused in man by prolonged inhalation of cotton dust. It will be recalled that in the operatives respiratory changes are not usually noticeable before they have passed through ten to twenty or more years' work in the cardroom. In view of the short life-time of experimental animals and the necessity of obtaining concrete results within a limited time, the conditions of the experiment had to be considerably intensified. A further difficulty consists in the fact that, as compared with man, all experimental animals possess noses far more highly developed and far better suited to filter off the dust from the air before it reaches the deeper air-passages.

It was decided to use guinea-pigs for the investigation; owing to their small size it is possible to subject a fair number of them to the same conditions, and, in addition, this animal is best used in any anaphylaxis experiments. In an attempt to induce mouth-breathing the guinea-pigs' noses were covered with strips of adhesive plaster or cotton wool soaked with mastisol during their stay in the dust-laden air. They soon learned, however, to scratch the material away, and therefore it was necessary to renew the covering at short intervals. It is doubtful for how much of the whole time of exposure to the dust the nose was completely occluded, but there is no doubt that some oral respiration was actually induced by this means.

The dust-cage consisted of a box, 18 in. long, 12 in. wide, 8 in. high. The two long sides were made of glass. Each short side was fitted with a $\frac{3}{4}$ in. brass tube, one for admitting the dusty air, the other for carrying it away; the latter was plugged with cotton wool to remove the dust from the air passing into the laboratory. The bottom of the cage was fitted with a removable tin plate on which the excrementa were collected. The upper edges of the four side walls ended in a tin gutter, $\frac{1}{4}$ in. wide and $\frac{3}{4}$ in. deep, which was filled with liquid paraffin. The cover of the cage was loose; it consisted of a plate of sheet metal, on the lower surface of which was fixed a $\frac{1}{2}$ in. ridge, which dipped into the paraffin when the cover was placed on the box. This afforded an air-tight seal. As the animals tended to huddle together in one corner of the cage, the inside was divided into three compartments by transverse plates of perforated zinc. Six animals at a time were exposed to the dust.

By means of a small electrically driven " AKO " blower air was driven in a steady current through a two-litre Wulff bottle containing the finely sieved cotton dust, and thence into the cage. The amount of air blown through the cage was about 25 cubic feet per hour, the amount of dust carried with it, apart from unavoidable losses in the connecting tubes, was about 5 g. per hour. The Wulff bottle was kept constantly agitated to prevent clumping of the dust. The bottle was held by four steel springs, fitted to a brass collar on its neck, in a recess in the horizontal plate of an L-shaped bracket. The upper end of the bracket could rotate to-and-fro in a nutatory manner by means of a pin-and-hole hinge. The lower end of the vertical plate was fitted with a steel plate kept in touch with a revolving cam by means of a strong spiral spring which pulled the horizontal plate of the bracket towards the bench. The cam revolved at the rate of 50 r.p.m. ; when its point touched the steel plate, the Wulff bottle was tilted, and when it passed the plate, the spring drew the bottle down again with a smart jerk* (Fig. 4, page 21).

In this manner 40 guinea-pigs were treated for 3–4 hours every weekday. The majority of the animals appeared to remain in good health, although at the end of each day's dusting they were thickly coated with dust. Their weight increased steadily and rapidly. Some of them, when placed in the cage, sneezed and coughed a little, but all soon got used to the treatment and appeared not to mind it. When taken out of the cage they usually breathed rapidly, but their first response on returning to their proper cages was to feed ravenously. In a few the fur grew patchy and some bald spots developed, but in the majority no changes of the skin occurred. Some of them developed a little local eczema in consequence of the plastering-up of their noses, but this cleared up rapidly after the plaster had been left off for a few days.

A few animals died intercurrently, one of Gaertner infection, which was prevalent in the laboratory at the time, and three of pneumonia (1 after 14, 1 after 16, 1 after 30 weeks). Three more animals died intercurrently without any noticeable post-mortem changes (1 after 2½, 1 after 3, and 1 after 8 weeks). The remaining animals were killed after varying intervals ranging from 3 to 36 weeks.

All the animals killed after at least two months' dusting showed pronounced macroscopical changes of the lungs. The pleural surface had remained smooth and glistening, but it was more or less deeply pitted and had a peculiar mottled greyish-black colour. This was particularly marked in animals which had been killed by cutting the throat : in controls the lungs were uniformly pale white, whilst in the dusted animals the greyish-black spots were in strong contrast to the white colour of the remainder of the lung. These changes are shown in Figs. 6 and 7. Usually, the discolouration was greatest along the lower and anterior borders of the middle and inferior lobes.

* The blower was supplied by the Scientific Glass-Blowing Co. ; the shaker was constructed by Messrs. Baskerville and Lindsay.

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ON HISTAMINE IN COTTON DUST, AND IN THE BLOOD OF COTTON WORKERS

BY

E. HAWORTH AND A. D. MACDONALD

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ON HISTAMINE IN COTTON DUST, AND IN THE BLOOD OF COTTON WORKERS

By E. HAWORTH and A. D. MACDONALD

From the Departments of Bacteriology and Pharmacology, the University of Manchester

(With 5 Figures in the Text)

Introduction

The possible relationship of respiratory disease in cotton workers to their employment, and in particular to the dusty conditions under which the strippers and grinders often operate in the card rooms, has long been a problem of considerable importance. In the past 6 years several relevant investigations have been carried out in these laboratories.

The presence of histamine, or of a "histamine-like substance"—so like it that for convenience we have long called it histamine—was detected in cotton-dust extracts, prepared from card-room dust, by Heap *et al.* (1932) by pharmacological methods. Such methods are of the order of one thousand times as sensitive as the most delicate of the known chemical tests, and also more specific. The further examination by Macdonald & Maitland (1934) of cotton, coir, and esparto-grass dust indicated that cotton dust was much richer in histamine than the others, and that in two dusts which were fractionated in accordance with particle size, the histamine was most plentiful in the finest fraction consisting of particles under 20μ in length.

These findings have been considerably amplified by Prausnitz (1936), whose report should be seen for a consideration of the whole problem, and a description of the classical symptoms of "stripper's asthma" in its various stages (p. 8). His method of histamine extraction, using larger scale apparatus, was less complete and gave a lower yield than the Soxhlet extraction previously employed, but was less open to possibilities that the histamine was not preformed. The yield he obtained was of the order of 0·003–0·02 mg. per 100 g. of husks, kernels, and delinted seed, but the card-room sliver was histamine-free. Much higher figures have been reported previously.

Prausnitz, in his report, concludes that "stripper's asthma" is a true allergic supersensitiveness to the protein of the dust, and that the role of histamine in causing the typical disease was doubtful. This paper presents work carried out along two lines: (*a*) the confirmation of the presence of histamine by its separation, purification, and chemical identification; and (*b*) the investigation of the blood histamine of card-room workers, and of controls.

It is believed that the results justify this further attention to the histamine side of the problem.

SEPARATION OF CRYSTALLINE HISTAMINE

Two methods have been followed by one of us (E. H.) for the chemical identification of the histamine. Most of the steps have been checked by biological tests.

(1) 10 kg. of fine cotton dust were boiled for 12 hours with 3 per cent HCl. Extraction of the concentrated filtrate with 2 parts of ether and 1 of 3 per cent HCl separated the tarry materials into the ether layer and the basic substances into the acid. The method of Best *et al.* (1926–7) for the extraction of histamine from ox liver was then applied, but it was found that the depressor substance had been absorbed on the precipitate formed when H_2SO_4 is added to give a 5 per cent solution prior to the addition of phosphotungstic acid, and on the crystals obtained by the concentration of this solution. The amount of depressor substance there, however, did not seem sufficient to make re-extraction of these two precipitates worth while; in all the numerous stages of the extraction, a certain proportion of the histamine is inevitably lost.

(2) A further 5·5 kg. of fine dust were similarly treated until after the basic lead acetate precipitate had been filtered off.

It was found at this stage that acidification of the solution with concentrated HCl gave a dense precipitate of small white crystals. After filtration, a portion was tested with H_2S for lead but none was present.

The acid solution was evaporated down when a few more crystals were deposited. These were filtered off and the filtrate taken to dryness *in vacuo*. Three portions of about 40 c.c. of 98·5 per cent alcohol were distilled off *in vacuo* to dry the residue completely and to remove most of the HCl. The residue was extracted with 98·5 per cent alcohol, made just acid to litmus with N H_2SO_4 by boiling under a reflux for $1\frac{1}{2}$ hours. The supernatant liquid was decanted off and filtered. This was repeated three times and at the end of the fourth extraction the whole contents of the flask were filtered. The combined filtrates were heated on a water-bath to remove the alcohol. Water was added to the residue and the H_2SO_4 removed as barium sulphate. The solution was still acid, so it was neutralized with NaOH and made just acid again with N H_2SO_4. The solution was concentrated and left for a week. At the end of this time one or two large crystals that looked like sodium sulphate had formed. These were filtered off and any sulphate ions removed as barium sulphate. The neutral filtrate was made up to a convenient volume—60 c.c.

10 c.c. of this were taken, 3 g. solid NaOH were added and the mixture extracted six times with redistilled amyl alcohol using 20 c.c. for each extraction. The addition of 3 g. solid NaOH to 10 c.c. of the concentrated solution gave a very heavy precipitate which made the separation long and difficult. It was found better to do the separation in a medium size centrifuge tube and after each extraction to spin it at a low rate for a few seconds on the centrifuge and to pipette off the supernatant liquid. The combined extracts were then extracted with N H_2SO_4 five times, using 20 c.c. for the first and 10 c.c. for

subsequent extractions. This was repeated until all the initial solution had been extracted. This method of amyl alcohol extraction was part of the extraction used by Koessler & Hanke (1920) for the quantitative colorimetric estimation of histamine in protein and protein-containing material. This removes the histamine from the histidine almost completely. The combined H_2SO_4 extracts were reduced in bulk and the H_2SO_4 removed as barium sulphate. A small portion of the concentrated H_2SO_4 extract was tested for histamine and found positive, using Hunter's (1928) modification of the Pauly reaction.

After the positive Pauly reaction, sufficient sodium sulphate solution was added to the solution to precipitate the excess barium completely and the mixture digested on the water-bath for 1 hour. The precipitate was filtered off and washed with hot water. The filtrate was reduced in bulk to about 50 c.c. and exactly neutralized with NaOH. It was then evaporated to dryness *in vacuo*.

The perfectly dry residue was treated with 30 c.c. of methyl alcohol and 1·5 g. caustic potash. The alkaline mixture was treated with 400 c.c. of redistilled chloroform and left in the ice-chest for a day, when it was filtered through a small folded filter paper and the residue washed with 400 c.c. of hot chloroform. A few drops of 37 per cent HCl were added to the chloroform extract and distilled *in vacuo* to remove the chloroform and methyl alcohol. Water was added to the residue and distilled off *in vacuo* to remove the methyl alcohol completely and most of the HCl.

The residue was taken up in a small amount of water and divided into two parts.

A saturated solution of sodium picrate was added to the first portion drop by drop. A cloudy precipitate formed, from which, on standing, drops of oil appeared on the bottom of the flask. More sodium picrate solution was added and the solution left to crystallize slowly. The oil solidified and small, hexagonal, pale yellow crystals formed on it. The mother liquor was pipetted from the crystals on to a watch-glass and allowed to crystallize. A pale yellow feathery-shaped crystal composed of small hexagonal plates was formed. This was carefully extracted from the mother liquor and dried first on filter paper and then in the hot-air oven. Some of the first set of crystals were also dried in the same manner and a melting-point was taken of each.

The first set of crystals which were contaminated with the solidified oil melted with decomposition at 205–210° C. The second set melted with decomposition at 237–239° C. This agrees quite well with the melting-point for histamine picrate given by Best *et al.* (1926–7). They give the melting-point of histamine dipicrate (recrystallized twice from water) as 241° C. with decomposition.

The crystals obtained were feathery and composed of small apparently monoclinic crystals. This is in agreement with the observations of Itallie & Steenhauer (1925), who describe histamine picrate as pale yellow feathery

crystals, and Takalasi *et al.* (1931), who describe histamine picrate as monoclinic and melting at 232–233° C. Unfortunately there was insufficient material for a recrystallization, or a mixed melting-point with pure histamine picrate.

To the second portion of the final extract a few drops of 37 per cent HCl were added and the solution allowed to crystallize. White crystals were obtained which melted with decomposition at 235–240° C. This agrees with the melting-point (233–240° C.) given for histamine hydrochloride by Klein & Boser (1932).

Here again the amount of hydrochloride obtained was insufficient to allow of a recrystallization and a mixed melting-point with pure histamine hydrochloride.

From the above results the substance in cotton-dust extracts which is pharmacologically the same as histamine is also chemically the same, as shown by the Pauly reaction and by the fact that its salts are identical in appearance and melting-point with those of histamine.

The biological detection of histamine in cotton dust thus receives chemical confirmation.

HISTAMINE IN BLOOD

A method for the estimation of histamine in blood was published recently by Barsoum & Gaddum (1935). The presence of histamine in whole blood cannot be demonstrated, but by their technique with Gaddum's modification, according to which concentrated HCl is substituted for normal acid, an isotonic and neutral solution can be obtained and its histamine content assayed on one or more biological indicators—an isolated segment of guinea-pig ileum in oxygenated Ringer-Tyrode, as suggested by Guggenheim & Löffler (1916), is probably as good and convenient as any, and we have used it in all cases.

Because of the small amounts of histamine which have to be estimated, a sensitive indicator in a small bath is desirable. We have used a bath for the gut which requires only 2 c.c. of Tyrode, and have found that with a sensitive muscle, such as that used for the record shown in Fig. 1, the response is measurably different in the sensitive range for increments of 0·005–0·01 c.c. of $H/5$ (a solution of histamine containing $0{\cdot}2\gamma$ per c.c.). Such a muscle is therefore sensitive to differences of $0{\cdot}001$–$0{\cdot}002\gamma$ H in the 2 c.c.—a change in concentration of the order of 10^{-9}.

It was felt that a comparison between the normal blood-histamine figures and those for card-room workers, many of whom are daily exposed to a histamine-containing dust—a dust which Prausnitz has shown to be so fine that ventilation, however much improved, cannot give complete protection against it—might yield interesting information.

Barsoum & Gaddum give the figures of 0·03 and $0{\cdot}04\gamma$ per c.c. (for convenience we prefer to take such figures in terms of γ per litre) as the histamine equivalent of human blood. A number of male university students were taken as normals, and in the first eight the blood histamine was found to vary between 21 and 63γ per litre. Such variation made it necessary to examine a con-

siderable series in order to know what limits might be regarded as normal. Over one hundred bloods were therefore assayed, and the results are given in

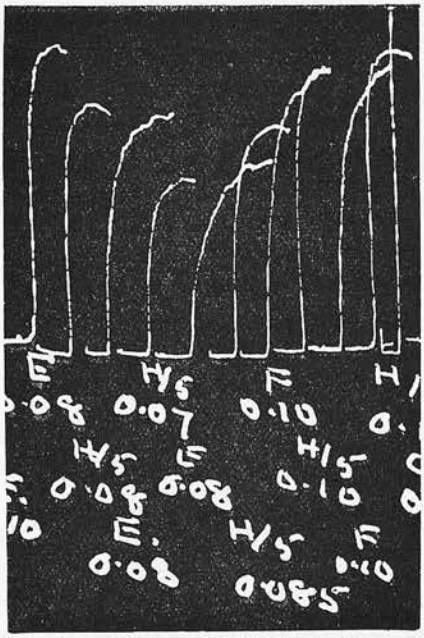

Fig. 1. Assay of blood histamine E (2·5 c.c., E represents 10 c.c. blood) against a histamine solution $H/5$ (0·2γ per c.c.) on sensitive segment of guinea-pig ileum. Comparing the first six contractions from left to right, E 0·08 > $H/5$ 0·08 > E 0·08 > $H/5$ 0·07 < E 0·08 < $H/5$ 0·085. From this we assume E 0·08 = $H/5$ 0·08, i.e. $E = H/5$, or 0·2γ per c.c., and the blood contains 0·05γ per c.c. or 50γ per litre.

the form of a distribution curve (Fig. 2). A few were re-examined at intervals, and these are tabulated (Table I) to show that, even when this interval is considerable, normal blood histamine does not vary greatly. Such a measure of agreement, with a fair range of blood histamines, gives the experimenter considerable confidence that he is measuring with fair accuracy a fairly constant blood constituent.

Table I. *Repeated estimations in normals*

Initials	Blood histamine in γ per litre: 1st estimation	2nd estimation	Interval days
G. R. C.	51	52	14
A. D. L.	21	21	23
G. K. T.	28	28	19
R. T. G.	70	67	19
D. B.	27	30	128
T. H. R.	38	33	23
S. E. G.	39	27	19
A. D. M.	19	19	5

The distribution curve (Fig. 2) was compiled during the collection of the figures. It suggested—especially at a middle stage of its preparation—that the histamine equivalent of a "normal" blood tended to fall into one of two

classes—between 20 and 40 or between 50 and 70γ per litre. Any such differentiation became less definite with increasing numbers of estimations, and it is improbable that a statistician would accept Fig. 2 as indicating the existence of two classes. It was felt, however, in many cases that one could foretell from the appearance of the donor whether his blood-histamine level would be high or not, but this, of course, does not guarantee the existence of two separable groups.

Distribution graphs for the histamine content of the blood

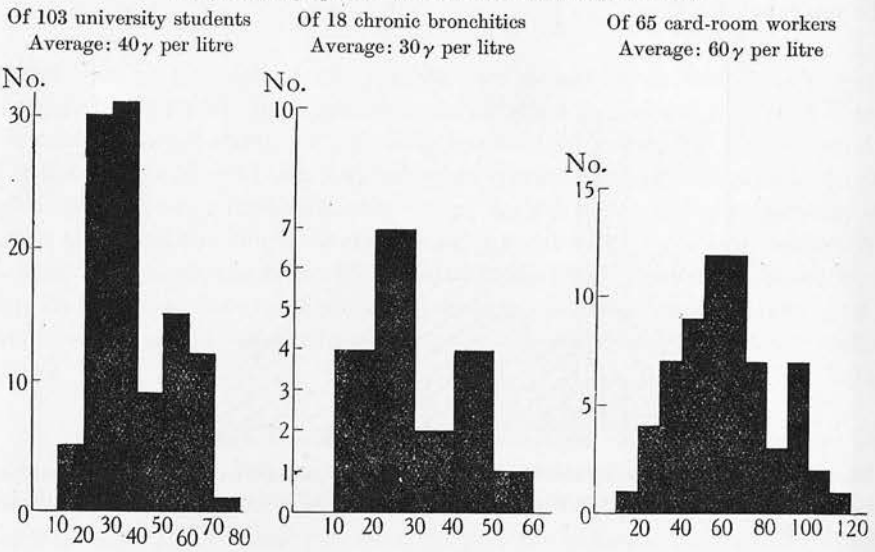

Fig. 2 Fig. 3 Fig. 4

University students obviously do not form an ideal control for card-room workers. They are younger, come from a more favoured section of the population, and lead very different lives from factory hands. The ideal control would be workers of similar ages from some other rather dusty occupation, but to get such in adequate numbers presented difficulties. We have, however, carried out tests on a number of hospitalized chronic bronchitics, older than the card-room workers, but whose respiratory disease was not directly attributable to dusty occupations. The distribution of the blood histamines in this group is shown in Fig. 3. The figures are not above those for students.

Through the kindness and co-operation of the officials of the Amalgamated Association of Blowing, Card, and Ring Room Operatives, blood samples for histamine assay were collected from over seventy card-room workers in the Bolton and Oldham areas. These men were by no means equally exposed to histamine-containing dust. Conditions vary with different cottons and different mills. Some had been at work on the day on which they were giving blood samples; others were on rather broken time, working only 3 days a week or less; many had been so incapacitated by respiratory disease that they had

been off work for some time. Some of this last section had made attempts to begin again but had been unequal to the work; others had tried to turn their hands to other occupations; still others were clearly unequal to any manual labour. Among the workers the stage of respiratory distress varied enormously, but scarcely one was free from at least some "tightness in the chest" and trouble on Monday afternoons; the worst were often late in arriving because of their difficulty in getting along from the mill, and especially up a stair; almost all were interested in our tests and feared that the dust would eventually be too much for them.

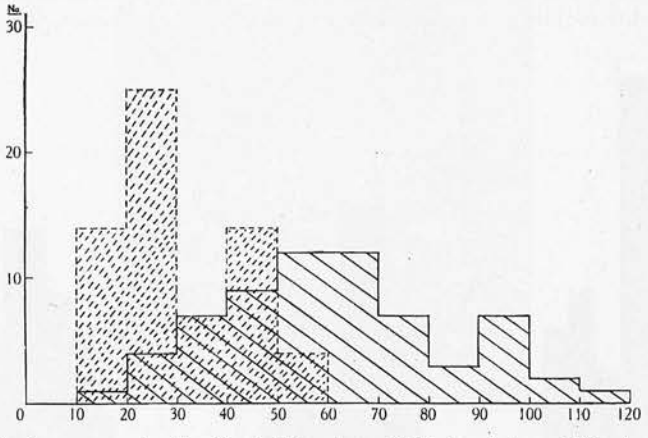

Fig. 5. Distribution curves for the blood histamines of 65 chronic bronchitics (calculated from Fig. 3) and the 65 card-room workers of Fig. 4. Only 25/65 of the range is common to the two groups.

The division of these operatives into such classes as

(*a*) working whole time in the card room,
(*b*) working part time there,
(*c*) working elsewhere, and
(*d*) not fit for work,

meant such reduction of the numbers in our groups as would make the figures obtained from them of doubtful significance. We have accordingly, rather regretfully, taken them as a single group—they are probably a reasonable cross-section of that portion of the population which has earned or is earning its living in card rooms. A study of the distribution figure for this cross-section (Fig. 4) leaves one in no doubt that there is very definitely raised blood histamine in card-room workers as compared with either university students or elderly chronic bronchitics. To emphasize this differentiation Figs. 3 and 4 are combined as Fig. 5, the chronic bronchitics of Fig. 3 being multiplied by a factor to make them equal in number to the card-room operatives. In Fig. 5 only about one-third of the field is common to the two groups; almost two-thirds of the group of card-room workers have a higher blood histamine than elderly chronic bronchitics.

A few of the card-room workers presented themselves for a second blood-

histamine assay. The two figures obtained for these and the intervals between the assays are set out in Table II. Without exception rather widely different

Table II. *Repeated estimations in card-room operatives*

Initials	Blood histamine in γ per litre		Interval days
	1st estimation	2nd estimation	
J. W.	63	33	90
J. H.	66	42	75
T. C.	56	95	27
W. H.	30	44	27
W. B.	58	75	75
W. R. B.	90	52	27

values were got for these men on the two occasions on which they were examined. Had such discrepancies been obtained in Table I we would have felt most unhappy about our technique or about the reliability of the method. Yet the routine of collection and assay had continued unaltered. While one is unwilling to base substantial theories on such a small series of patients it seems clear that the blood-histamine level is much more variable in card-room workers than in our controls. Such variation is probably quite significant, and may be related to the extent of the exposure to the histamine-containing dust, and to the interval between the collection of the blood and the last asthmatic attack.

Discussion

In the light of the Prausnitz report, one might regard the presence of histamine in cotton dust as a mere incidental to card-room respiratory disability—possibly almost as an unfortunate red herring innocently drawn across the trail in 1932. The confirmation of the presence of histamine in the dust and the probable presence in the blood of the card-room worker of more histamine than is usually found in human blood make one hesitate to suggest that the protein alone is responsible, especially when we remember the possible role of histamine in allergic phenomena.

Daly *et al.* (1935) have brought forward a great deal of evidence that a histamine-like substance is released from the perfused lungs of the sensitized guinea-pig in anaphylactic shock, and corroborating assays of this substance against histamine on a variety of biological indicators—cat's blood pressure, guinea-pig ileum and guinea-pig lung, yield "strong presumptive evidence that the substance released from the lungs is histamine". May it not be that "stripper's asthma" differs from most asthma—as it certainly does—in that the two factors are present, absorption of histamine and of the allergen which "touches it off"? On some such hypothesis it might even be possible to develop a working hypothesis for the difficult "Monday sickness". In the week-end, in the absence of exposure to the specific protein, there may well be some accumulation of the histamine in lung tissues—from the blood and/or the alveoli—and this may be liberated by Monday's contact with allergen. During the rest of the week there may be less accumulation, and less severe symptoms follow. This, at any rate, may be more acceptable than Prausnitz's

suggestion that the card-room worker, having breathed deeply of uncontaminated air in the week-end, has to learn painfully each Monday to breathe shallowly while at work. The suffering experienced by many workers every Monday would surely make such repeated lessons unnecessary.

Much work on such points needs to be done. It would be interesting to follow the variations in blood histamine in selected sufferers from "Monday sickness" in some detail. The figures we have given for bronchitics require extension, and a fresh series for asthmatics not exposed to cotton dust is needed. It is hoped to undertake further work on these lines in the near future.

SUMMARY

Crystalline histamine picrate and hydrochloride have been prepared from cotton dust. The melting-points and crystalline structure found are in agreement with those obtained by other workers.

The blood histamines in sixty-five card-room workers have been estimated and compared with the figures found in 103 students and eighteen elderly chronic bronchitics. The figures for the three groups are shown in distribution figures, and the card-room workers have, on an average, a higher blood histamine than both groups of controls. It has, however, to be admitted that the differentiation of card-room asthma from other respiratory diseases on the basis of the level of blood histamine cannot be guaranteed in individuals.

ACKNOWLEDGEMENTS. It is a pleasure to put on record our sincere thanks to all who have so cheerfully given samples of their blood for these estimations, to the Medical Superintendents of Crumpsall and Withington Hospitals for facilities in their institutions, and especially to the officials of the Amalgamated Association of Blowing, Card, and Ring Room Operatives in Manchester, Bolton and Oldham for their interest and courtesy in finding us members for examination.

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(26).

THE HISTAMINE-LIKE ACTIVITY OF BLOOD

BY

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THE HISTAMINE-LIKE ACTIVITY OF BLOOD

In blood there are many substances which have been isolated in pure form and for which chemical methods of estimation have been devised. These substances are present in relatively high concentration. They are, for the most part, devoid of marked pharmacological activity, and their quantitative estimation is, in many instances, a routine clinical procedure. In addition to these presumably *inert* constituents there are, in blood, a number of substances present in minute quantities but possessing exceedingly powerful physiological properties, which may be regarded as the *active* components of blood.

The presence of the active constituents may be masked by the manner in which they are held in the blood. The changes which occur in blood on clotting (O'Connor 1912), on injuring or hæmolysing the cells (Phemister and Hardy 1927), or even on allowing drawn blood to stand may serve to free these substances in a form in which they can produce their physiological effects. Chemical treatment of blood may also liberate active constituents. The use of such methods led to the demonstration of a histamine-like substance which may be extracted from blood. The separation of histamine from the complex mixture of active components, which under certain circumstances may be found in blood, has marked an advance in our knowledge of the pharmacological properties of blood.

Histamine, it may be remembered, has remarkable constrictor effects on plain muscle and dilator effects on the capillaries, and has long attracted attention in connexion with the toxæmia theory of shock.

EXTRACTION AND QUANTITATIVE ESTIMATION OF THE HISTAMINE-LIKE SUBSTANCE

Following the demonstration of the potent biological properties of histamine (Dale and Laidlaw 1910) various methods were devised for its quantitative estimation. No purely chemical method has been found sufficiently sensitive to estimate the small quantities of histamine believed to be present in blood. Methods involving a preliminary purification of the active substance, followed by a quantitative assay, by means of one or more of the physiological responses to histamine, have been successfully applied to blood. The method recently developed by Barsoum and Gaddum (1935a) has been most widely used. It

involves the separation of the active substance and its assay on a segment of guinea-pig ileum. Recently Code (1937a) has subjected the method to analysis, and, upon the basis of estimations in which known amounts of pure histamine were added to water and to blood, has developed an improved and simplified technique.

It is important to draw attention to the method of expressing the histamine equivalent of blood. Various preparations of pure histamine may be used as a standard against which to assay the activity of the blood extracts. The histamine base content of all such preparations may be calculated and the expression of results in terms of the base provides a satisfactory constant and allows a ready comparison of data from different sources. The quantity of histamine-like substance has generally been stated in terms of γ histamine per c.cm. ($1\ \gamma = 0{\cdot}001$ mg.).

NORMAL HUMAN BLOOD-HISTAMINE EQUIVALENTS

Barsoum and Gaddum (1935a) gave a few figures for normal human blood. Haworth and Macdonald (1937) have published figures for 103 university students believed to be healthy. The figures range from $0{\cdot}018$ to $0{\cdot}078\ \gamma$ per c.cm. and average $0{\cdot}04\ \gamma$ per c.cm. They suggested the possibility of the existence of two types of "normal"—one averaging $0{\cdot}03\ \gamma$ and the other $0{\cdot}06\ \gamma$ per c.cm. Their repeated observations at intervals varying from 5 to 128 days indicated that in a healthy person the blood histamine varies little from time to time.

LIBERATION OF HISTAMINE-LIKE SUBSTANCE DURING REACTIVE HYPERÆMIA

The experiments of Lewis and Grant (1925–26) indicated that reactive hyperæmia was due to chemical substances accumulated in the limb during the period of obstructed circulation. Barsoum and Gaddum (1935b) estimated the histamine equivalent of femoral venous and arterial blood, before and after periods of circulatory arrest, in the hind limbs of anæsthetised dogs. Immediately after 10 or 15 minutes' ischæmia, the venous histamine equivalent was approximately four times that of arterial blood. The venous blood value then sharply declined and 5–7 minutes after release of the circulation it had returned to the arterial value. Continuous reperfusion of defibrinated blood through the hind limb raised the histamine equivalent of the circulating blood several-fold. Reoxygenation of the blood by lungs placed in the perfusion circuit resulted in a return to the original histamine equivalent. When the lungs included in the circuit were made to respire air containing 10–20 per cent. of carbon dioxide the whole blood-histamine equivalent again rose. The effect of anoxæmia and increased

CO_2 tensions on the histamine equivalent of blood entirely removed from the body was, apparently, not investigated.

When the whole blood-histamine equivalent is increased from any cause it is important to ascertain which portion of the blood contains the increased activity. If the active substance be present in the cells it may not be free to produce its physiological effects. Barsoum and Smirk (1936a) have studied the concentration of the histamine-like substance in the plasma and red cells of venous blood from normal arms and arms in a state of reactive hyperæmia. In their ten experiments the histamine equivalent of the plasma was increased from an average value of 0·006 γ per c.cm. to an average value of 0·025 γ per c.cm. while the values for the corpuscles showed a comparatively slight increase. It is their belief that the liberation of this histamine-like substance during circulatory arrest accounts, at least in part, for the hyperæmia.

PRODUCTION OF HISTAMINE-LIKE ACTIVITY BY CONTRACTING MUSCLE

The mechanism involved in the production of the hyperæmia which follows muscular contraction has for years interested investigators. Anrep and Barsoum (1935a) have estimated the histamine-like substance in arterial and venous blood during contraction of the dog's gastrocnemius muscle. A conspicuous increase in the histamine-like activity of extracts of the venous blood was associated with muscular contraction, offering a possible explanation of the increased blood flow. The histamine equivalents of plasma and red cells during the period of hyperæmia were not recorded. It would be interesting to know the effect, on the muscle circulation, of pure histamine added to the blood in the concentration found during muscular contraction. Muscle perfused with a protein-free physiological fluid might possibly enable the active substance to be recognised without the necessity of a chemical extraction.

TRAUMATIC SHOCK AND THE HISTAMINE EQUIVALENT OF BLOOD

Many attempts have been made to discount the toxæmia theory of shock and replace it with other explanations because of the difficulty of demonstrating the presence of active substances in the blood in shock (Holt and Macdonald 1934, O'Shaughnessy and Slome 1934–35). Further investigations have been stimulated by the development of methods for estimating the blood-histamine equivalent.

Holt and Macdonald (1936) estimated blood-histamine equivalents in cats before and after shock-

producing trauma to a hind limb. Even when the second sample was taken after shock was fully established and the animal moribund there was no demonstrable rise in its blood-histamine equivalent.

Minard (1937) has however compared the histamine contents of the venous blood from traumatised and normal limbs, and finds an increase in the blood from the shock-producing area which averages 80 per cent. of the normal content. He agrees that the general level of blood histamine is not raised in acute shock. This may be because of the rapidity with which histamine can normally be "fixed" by the tissues. No account of more chronic experiments on traumatic shock has yet appeared, and it is hard to say what significance, if any, must be attributed to the production of histamine-like substances in traumatised tissues. Recent work on burns, however, indicates that the toxæmia theory will require further careful investigation and consideration.

EFFECT OF CUTANEOUS BURNS ON THE BLOOD-HISTAMINE EQUIVALENT

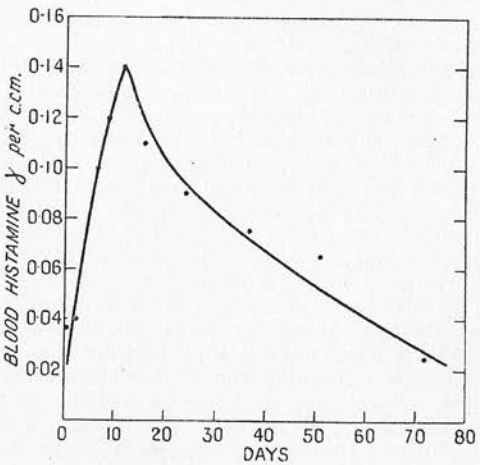
Barsoum and Gaddum (1936) have reported investigations of the blood-histamine changes following extensive cutaneous burns. Their cases, some of which terminated fatally, received the usual tannic acid treatment. The blood histamine rose to four or more times its normal level on the fourth to the sixth day—the usual time for such patients to develop secondary shock. As a rule the blood-histamine equivalent slowly settles again to its normal value. The conclusion was reached that the rise in blood histamine is not likely to be entirely attributable to production in the burnt skin—it may be secondary to pathological changes in the liver and kidneys.

In a woman with severe burns of both legs and buttocks one of us followed up the blood histamine throughout her long illness. She had "no tannic acid treatment because of severe shock and sepsis," but morphine, glucose-salines, and a blood transfusion. On the day after the burn the blood-histamine equivalent was 0·037 γ per c.cm. This figure rose slowly to 0·14 γ on the twelfth day, and then gradually subsided but was not normal till two months later. The estimations are plotted on the accompanying Figure, and yield a reasonably smooth curve. It would clearly be interesting to know whether the active substance is present in the plasma, and thus free to produce its physiological effects, or fixed in the cells.

ASTHMA AND THE HISTAMINE-LIKE SUBSTANCE IN BLOOD

The action of histamine on the bronchi has led to its study in connexion with asthma. Haworth and

Macdonald (1937) have carried out blood-histamine estimations on a series of 65 card-room workers, who have to contend with dusty working conditions and with a dust which has been shown to contain histamine. Most but not all of these suffered to some extent from "stripper's asthma." It was not surprising, therefore, to find that their blood-histamine equivalent was raised to an average of 0·06 γ per c.cm. The highest figure found in any of these workers was



Results of ten duplicate estimations of blood histamine in a patient with severe burns.

0·12 γ per c.cm.—a figure far exceeded in certain other diseases.

Unfortunately a series of figures for asthmatics not similarly exposed to a histamine-containing dust has not yet been obtained, but in 18 elderly chronic bronchitics the figures found were all within the normal range, averaging 0·03 γ per c.cm.

SOURCE IN BLOOD OF THE HISTAMINE-LIKE SUBSTANCE

Quite apart from additional histamine-like activity which may appear in extracts of blood under various physiological and abnormal conditions, it is desirable to have some understanding of the source in normal blood of the active constituent. The observation that normal blood yields the active substance invites the question, in what manner is the activity held in blood ?

In their first publication, Barsoum and Gaddum (1935a) reported that the histamine equivalent of the cells of rabbit blood was about six times that of the plasma. Anrep and Barsoum (1935b) in their

investigation of the distribution of the histamine between plasma and red cells, found the corpuscle/plasma ratio in rabbit's blood to be well above 10 : 1 and sometimes as high as 18 : 1.

The distribution of the histamine-like substance of human blood has been studied by Barsoum and Smirk (1936a and b). Their data indicate that the concentration of the active substance is about 10–60 times higher in the red cells than in the plasma of normal blood.

Recently Code (1937b) has studied the source of the active substance in normal blood. Throughout the investigation an effort was made to maintain the normal condition of the blood. Anoxæmia was avoided and where possible no anticoagulants were used. Pure red cells were sampled from the bottom of the centrifuge tube. Under such conditions the plasma and red cells were found to contain little of the histamine-like activity. When the quantities of histamine-like substance estimated to be present in the plasma and red cells of 10 c.cm. of blood were added together, only 10–12 per cent. of the whole blood activity had been recovered. The only portion of the centrifuged blood not included in these samples was the layer which separates out between the plasma and red cells containing the white blood-cells and platelets ("white cell layer"). The activity unaccounted for in the red cells and plasma was found in the white cell layer. In man, rabbit, dog, horse, bullock, and goat, 70–100 per cent. of the histamine-like substance extractable from whole blood was contained in the white cell layer of normal unclotted centrifuged blood.

In a more detailed study of rabbit blood, it was observed that clotting liberated the active substance so that it appeared quantitatively in the serum. The activity therefore belongs to that group of substances which are freed from cellular constituents of blood on clot formation (O'Connor 1912). Clotting of blood does not in itself form the histamine-like substance, since clot formation does not alter the whole blood-histamine equivalent. Coagulation of blood simply allows the activity to diffuse from the white cell layer into the fluid portion of the blood.

HISTAMINE-LIKE ACTIVITY OF WHITE BLOOD-CELLS

A further investigation was undertaken in the hope of determining which of the constituents of the white cell layer gave rise to the histamine-like activity (Code 1937c). Early in this study it became evident that valuable information could be obtained from the study of the blood of patients having certain blood dyscrasies. Macdonald, who was in close touch with the progress of this investigation, carried out a series of

estimations on the blood of such patients. The results by Macdonald in Manchester and Code in London are incorporated in Tables I–III. The fact that these observations were made quite separately adds considerably to their significance.

The observation that when blood clots, the activity is liberated from the white cell layer into the serum, focused attention upon the platelets as the most likely source of the active substance. Practically pure platelets were prepared from horse and human blood. Extraction of these platelets yielded little or

TABLE I

Histamine Equivalent of the Blood of Patients with Lymphatic Leukæmia

Patient	Total white cell count	Lymphocytes (per cent.)	Histamine (γ per c.cm.)
R. C.	157,000	94	0·021
W. J.	228,000	98	0·050 0·052
M. E.	22,300	96	0·031 0·027
H. S.	31,400	87	0·100
H. E.	110,000	95	0·086
C. B.	413,000	99	0·062 0·062

none of the active substance (Code 1937c). It was, therefore, necessary to investigate separately the different types of white blood-cells.

Non-granular series of cells.—In order to determine the histamine equivalent of lymphocytes, extracts were prepared from lymph glands and from the blood of lymphatic leukæmia patients. Extraction of lymph glands yielded no significant amount of the histamine-like activity. The blood of lymphatic leukæmia patients was found to give a normal histamine-equivalent value (Table I).

Monocytes were obtained from sterile pleural exudates produced in response to oil placed in the pleural space. Extracts of monocytes under these conditions were for practical purposes histamine-free.

Granular series of cells.—If the platelets, lymphocytes, and monocytes do not contain the histamine-like activity, the granular series of cells must be rich in the active substance. To test the truth of this the blood of patients with myeloid leukæmia was studied. Results obtained by Macdonald and Code demonstrated an enormous increase in the whole blood-histamine equivalent of myeloid leukæmia patients

(Table II). Values up to 300 times the normal histamine equivalent were encountered.

This increase in blood histamine is not to be attributed, apparently, to the severe anæmia which is usually found in myeloid leukæmia, for in three cases of pernicious anæmia in which the anæmia was similarly advanced (a red cell count of about two millions) the blood-histamine equivalents fell within the normal limits (0·06, 0·06, and 0·03 γ per c.cm.). Further, when the myeloid patient receives X ray therapy, there is a fall in the number of white cells and a fall in the histamine equivalent. One would

TABLE II

Histamine Equivalent of Blood of Patients with Myeloid Leukæmia

Patient	X ray therapy	Total white cells	Eosinophils (per cent.)	Granular (per cent.)	Histamine (γ per c.cm.)
B.	Before.	257,200	9·8	97	17·5 & 15·2
	After.	74,800	7·0	90	6·3
H. A.	Before.	176,800	2·6	97	3·5 & 2·5
	After.	167,200	0·5 (10·5)	95	1·8
H.	After.	95,000	(1·7)	95	1·7 & 1·5
C.	After.	100,000	?	92	15·0 & 14·0
P.	Before.	224,000	(3)	99	3·1

wish to investigate a longer series of these patients at more frequent intervals in the hope of establishing some correlation on these lines.

Further experiments were then carried out with the aim of gaining some insight into the possible type of cell of the granular series containing the active substance.

Extracts of dog blood often contain no significant amount of histamine-like activity whereas those obtained from rabbit blood are relatively rich in it (Code 1937b). Histological comparison of smears of dog blood having no activity and rabbit blood rich in the active substance might be expected to indicate some striking difference. The cytoplasm of the polymorphonuclear cells of the dog is almost devoid of stained material and relatively few eosinophils are present. In contrast to this the polymorphonuclear cells of rabbit blood contain eosinophil granules. This is the normal appearance of rabbit blood and the cells have been referred to by hæmatologists as pseudo-eosinophils.

With this clue experiments were carried out by Code (1937c) in an effort to produce eosinophilia in dogs. Following the repeated oral administration of

Nirvanol the eosinophil percentage in the blood rose, and associated with this was an increase in the histamine activity of the blood. The appearance of histamine activity in the blood did not exactly parallel the eosinophil count. The experiment was indicative but not beyond criticism. Results obtained from the blood of patients with an eosinophilia have been more conclusive. With one exception a 9 per cent. or greater eosinophilia was accompanied by an increase in the whole blood-histamine equivalent (Table III). Again where repeated estimations have been possible

TABLE III

Histamine Equivalents of the Blood of Patients with Eosinophilia (not Myeloid Leukæmia)

Patient	Disease	Date	Eosinophils (per cent.)	Histamine (γ per c.cm.)
P. E.	Parasite ?	21/12/36	3000	0·50
		8/1/37	1850	0·44
		23/3/37	2010	0·51
		15/6/37	1260	0·34
W. K.	Splenic anæmia after splenectomy.	13/1/37	1230	0·19
		22/2/37	980	0·15
C. S.	?	12/2/37	960	0·15
		22/2/37	1090	0·14
S. E.	Parasite.	11/3/37	650	1·00
	Parasite removed.	22/3/37	1470	0·18
C. U.	Pemphigus.	21/4/37	1900	0·04

it has as a rule been found that in response to treatment the number of eosinophils and the histamine equivalent decrease together (S. E. in Table III is an apparent exception).

ISOLATION OF HISTAMINE FROM BLOOD

The methods involved in the demonstration of a histamine-like constituent of blood were open to two criticisms. Firstly, it was possible that some or all of the histamine activity was due to a secondary reaction during the rather drastic process of extraction and that histamine was not present as such in blood. Secondly, the quantities of histamine present in routine extracts of blood are so small that they allow only pharmacological identification. Rigid proof that the active substance was histamine could only be obtained by its chemical isolation.

The experiments of Code and Ing (1937) showed that the substance responsible for the histamine-like activity was present in the white cells and was not produced by the process of extraction. Without the use of drastic chemical methods they have isolated the active substance in pure crystalline form from

the white cells of rabbit's blood and have identified it as histamine. Histamine apparently may therefore be regarded as a normal constituent of the white blood-cells.

DISCUSSION

Throughout this review we have endeavoured to maintain a tone of restrained interpretation. We feel that this should be rigidly applied to the results just reported. The enormous increase in the histamine equivalent of the blood of myeloid leukæmia patients invites speculation. Too little is at present known about the meaning of the histamine-like activity of blood to justify the weaving of theories, however attractive. Experiments so far have shown that almost all the increase in histamine (95 per cent.) in the unclotted blood in myeloid leukæmia is fixed in the white cell layer. The fact of the presence in the white cells of this relatively large amount of very active substance is interesting enough, and it seems unlikely that the abnormal content in the circulating blood is without significance.

We are greatly indebted to all who have given us facilities and help in the work here recorded, particularly to Dr. J. F. Wilkinson, director of clinical research in the Royal Infirmary, Manchester, and his staff, for samples from anæmic patients, and to Mr. Herbert Broadley of the university department of pharmacy for his help in the preparation of these samples for assay.

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The action of the intercostal muscles. (*Preliminary note.*)

By E. Sharpey Schafer and A. D. Macdonald.

In an anæsthetised (or decerebrated) animal (dog, cat, rabbit) with respiration maintained by positive ventilation a complete hoop—including two adjacent ribs and rib cartilages, the intervening musculi intercostales and intercartilagines and the segment of the sternum to which the two ribs are attached—is isolated from the rest of the thorax, except at the attachment of the ribs to the vertebral column, by cutting through all muscular attachments above and below the hoop, the isolation being completed by severing the muscles right down to the spine. The nervous and vascular supply of the muscles of the hoop are not interfered with and due precautions are taken to maintain the temperature both of the animal's body and of the isolated thoracic hoop.

If now the artificial respiration is diminished the natural movements of respiration are resumed and the isolated hoop moves forward in inspiration with those ribs above and below it which were left *in situ* and also with the descent of the diaphragm, and backward in expiration with the backward movement of the ribs left *in situ* and with the ascent of the diaphragm (contraction of abdominal muscles).

On examining the preparation the musculi intercartilagines can be distinctly seen and felt to be contracting with inspiration and relaxing with expiration. But it requires closer observation to detect contraction of the external intercostals, since they appear not to contract as a whole, except perhaps in extreme dyspnœa. And if the fibres of the muscular sheet are only contracting here and there it is difficult to detect their movement amongst the many resting fibres. Nevertheless it is sometimes distinct. And that they are always able by themselves without the assistance of the intercartilagines to bring forward (raise) the ribs is shown by the fact that this movement still occurs after the intercartilagines are cut through. In this case the movement must be wholly due to the action of the external intercostals. For the internal intercostals cannot —owing to the direction of their fibres and the fact that they are put on the stretch when the ribs are moved forward—aid in this movement. On the contrary, they oppose it by their elasticity. Whether they take an active part in the backward or rib-depressing movement is difficult to determine. We have been unable to see or record such active contraction, but unless it were strong and involved all the fibres of any part

of the muscular sheet, it would be difficult to detect, or to differentiate from the passive retraction which results from the fibres having been put on the stretch during the forward movement of the ribs.

We had supposed it would be easily possible to resolve this question of the active participation of the internal intercostals in the expiratory movement of the ribs by the employment of the string galvanometer, but have met with unexpected difficulties in applying that method, and have not yet succeeded in entirely overcoming these difficulties.

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November 14th, 1931.

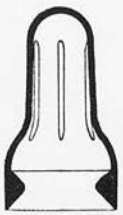
AN IMPROVED TEAT FOR INFANTS' FEEDING-BOTTLES

Dr. A. D. MACDONALD (Department of Pharmacology, University of Manchester) writes:

The rubber teats commonly used on infants' feeding-bottles are made by the repeated immersion of a suitably shaped mould in a rubber solution, and the teat, when a suitable thickness is obtained, is "cold-vulcanized"—a process which involves the exposure of the article to the action of sulphur monochloride, either as a vapour or in solution. Such treatment produces a certain hardness and resilience. The whole process is slow, and the glass moulds require frequent replacement. It is also unsatisfactory, in that it is not particularly healthy for the workers; indeed, the manufacturer who uses it is required to obey rather stringent Home Office regulations. The cold-vulcanized rubber teat is not an altogether satisfactory makeshift; in France its sale is forbidden by law, but certain loopholes leave it possible for the regulations to be evaded on technical grounds.

The laboratory staff of Messrs. Macinlop, Ltd., Manchester, have been experimenting during the past year on the production of a moulded heat-vulcanized teat which would be free from the more serious objections which characterize the standard cold-vulcanized patterns. I have acted in an advisory capacity during the development of the moulded teat, and subjected the teat to a series of laboratory tests. It is clearly important to ascertain that the processes of manufacture do not introduce into the rubber any harmful substance.

For the production of a heat-vulcanized article the rubber is first mixed with a carefully controlled proportion of sulphur and subsidiary ingredients. The heat treatment is usually accomplished by inserting a weighed quantity of the mixed or "compounded" rubber in a steel mould, which is placed under hydraulic pressure between the platens of a steam-heated press. The degree of vulcanization is determined in part by the composition of the mixed rubber, and also by the temperature and duration of the heat treatment. In contrast to cold vulcanization, which is largely a surface treatment, heat vulcanization progresses uniformly throughout the mass, so that all portions of the finished article have the same characteristics. In addition to careful selection of the compound and all raw materials used, manufacturing operations must be carried out with strict attention to cleanliness, and the finished moulded teats are finally sterilized before wrapping.



In all my observations the new teats have been compared with those marketed by a well-known firm, and known to be manufactured by the older process. When new and unboiled the moulded teat is not quite as translucent as the cold-vulcanized article. It feels a little firmer and more substantial, and is free from any stickiness, which cannot always be claimed for the other. In rough tests

of toughness and elasticity there is little to choose between them.

Most important of the advantages that can be claimed for the moulded teat is that it can be sterilized repeatedly by boiling in water. I have boiled one for four days of about eight hours, without apparent deterioration. The cold-vulcanized teat, after thirty minutes' boiling, generally loses resilience, and becomes cracked and rough. These cracks, if the teats are used thereafter, might well be irritant, and make strict cleanliness difficult.

Milk is not affected in reaction, as tested by indicators, nor in palatability, when the new teat is immersed in it. Indeed, I have pumped a small quantity of milk through a teat repeatedly throughout an hour, and the appearance, reaction, and flavour of the milk are unaffected. When a teat has been immersed in milk for twenty-four hours the flavour of the milk is impaired, becoming " rubbery " ; but this is an academic objection, and applies equally to the cold-vulcanized product.

I have applied rubber strips, cut from the teats, to the skin of the forearm and axilla, retaining them in place by strips of zinc-oxide plaster. No reaction whatever was provoked in the skin by the heat-vulcanized rubber ; indeed, the zinc-oxide plaster produced a slight erythema, from which the rubber had shielded the area it covered.

Through the kindness of Dr. Catherine Chisholm, the moulded teats were then given a thorough clinical trial. On her recommendation they were lengthened, so that they could reach and stimulate the palate and dorsum of the tongue, if required, and thus produce more powerful suction. The shoulders were further strengthened, and the retaining ring improved, so that there is no risk of the infant sucking or pulling the teat from the bottle. For bottles with openings at either end a suitable valve of similar material has been moulded. It is believed that these teats will shortly be available for general use.

(29)

The Assay of Digitalis by the Cat Method

BY

A. D. MACDONALD

AND

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(*From the Department of Pharmacology, University of Manchester,*)

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THE ASSAY OF DIGITALIS BY THE CAT METHOD.

By A. D. MACDONALD and WALTER SCHLAPP.
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Received 30th May, 1930.

The cat method of assaying digitalis preparations, originally introduced by Hatcher and Brody,[2] has now been adopted as standard by a number of pharmacopœias. The experience gained in its use by various workers, especially by de Lind van Wijngaarden,[4] has resulted in the establishment of a formula by which the number of separate determinations required for an assay of any given degree of accuracy may be ascertained. The necessity for such a formula arises from the variations which occur between separate determinations of the lethal dose of a given preparation. That the variations are considerable is well known among those who are accustomed to use the method, and ample evidence thereof is to be found in the literature of the subject.

A certain amount of variation between different cats is, of course, only to be expected on biological grounds, but it seemed to us that the use of ether as an anæsthetic might be a factor contributing to irregularity, and that an improvement in the figures might be obtained by abandoning the practice of assaying one poisonous substance upon an animal already under the influence of another. Further, the action of digitalis on the mammalian heart is a complex one, the direct action on the heart being accompanied by the action on the medulla. Without going into the question of the relative importance of these two actions, it is probably better for purposes of assay to eliminate one of them, which is easily accomplished by using a spinal preparation. We have, therefore, carried out a number of assays upon spinal cats from which the ether has been ventilated, and the results encourage the belief that this modification of the cat method increases its accuracy considerably.

METHOD.

In accordance with van Wijngaarden's[4] suggestion we have selected for this work cats weighing between 1·7 and 2·7 kgm., avoiding those advanced in pregnancy and those in lactation.

The animal is anæsthetised with ether and its weight noted; the external jugular veins which have been exposed by the removal of the skin from the front of the neck, are ligated; the carotid sheaths are found and ligatured as a whole, the nerves on each side being then dissected out, and severed. The trachea is opened and the usual T-shaped tube tied in. The skin at the back of the neck is next removed and a decerebration clamp modelled on that devised by McDowall[3] is applied around the body of the second cervical vertebra, so as to occlude the vertebral arteries. The successful accomplishment of this is manifested by the disturbance, followed by the cessation, of natural respiration, and from now on artificial respiration from a pump is employed. When the degree of insufflation has been adjusted, the head is fully flexed and the muscles of the back of the neck are divided in a transverse direction down to the occipital bone. The occipito-atlantoid ligament is then exposed and on cutting through it the cord in its membranes becomes visible. This is now cut through and the venous ooze from the head stopped by pushing several pieces of cotton wool through the *foramen magnum*, a procedure which at the same time completes the destruction of the brain. The animal is now tied down on its back and the insufflation finally adjusted. A thermometer is inserted among the muscles of the neck beside the trachea and the animal left for not less than four hours, the

TABLE I.—ASSAYS ON SPINAL CATS. (Four Tinctures.)

Experiment No.	I.		II.		III.		IV.	
	Lethal Dose, 5 per cent. Tincture c.cs. per kgm.	Percentage of Average.	Lethal Dose, 5 per cent. Tincture c.cs. per kgm.	Percentage of Average.	Lethal Dose, 5 per cent. Tincture c.cs. per kgm.	Percentage of Average.	Lethal Dose, 5 per cent. Tincture c.cs. per kgm.	Percentage of Average.
1	18·4	90	26·1	99	25·0	95	24·2	101
2	19·5	96	27·1	102	27·9	106	22·9	95
3	20 3	100	28·6	108	28·7	109	24·0	100
4	20·3	100	26·2	99	25·3	96	23·4	97
5	22·8	112	24·5	92	24·7	94	25·7	107
6	21·3	104	26·6	100	26·5	100	—	—
Average	20·4		26·5		26·4		24·0	
Standard Deviation of Average: expressed as percentage of Average.	2·7 per cent.		1·9 per cent.		2·3 per cent.		1·7 per cent.	
Greatest Deviation in Series.	12 per cent.		8 per cent.		9 per cent.		7 per cent.	

heater being adjusted in such a way as to keep the temperature constant at about 37° C.

After the lapse of this period a cannula for recording blood-pressure is tied into the left carotid artery; a cannula connected to a burette fitted with a Mariotte tube, and ready filled with the solution to be assayed, is then tied into the right external jugular vein and when the time has been noted the infusion is begun by releasing the screw clip. For an approximately standard tincture, we find it convenient to estimate the desirable rate of inflow so that the cat will receive 20 c.c. /kgm. of the 5 per cent. dilution of the tincture in thirty minutes. In our experience the rate of inflow shows a general tendency to become slower, probably owing to alterations in the venous pressure. We make the necessary small adjustments of the inflow, which is observed every minute or every two minutes, by varying the level of the burette, which is fixed to a Palmer "Large Adjustable Screw" stand. We record the blood-pressure throughout the experiment; usually it rises steadily until some ten to fifteen minutes before the end when obvious cardiac irregularity sets in, followed by a gradual decrease

TABLE II.—ASSAYS ON ETHERISED CATS. (Three Tinctures.)
I & II. FROM J.H.B. III. BY A.D.M. & W.S.

Experiment No.	I.		II.		III.	
	Lethal Dose, 5 per cent Tincture c.cs. per kgm.	Percentage of Average.	Lethal Dose, 5 per cent. Tincture c.cs. per kgm.	Percentage of Average.	Lethal Dose, 5 per cent. Tincture c.cs. per kgm.	Percentage of Average.
1	14·5	91	13·91	80	20·0	96
2	16·9	106	16·35	94	25·0	120
3	17·9	112	16·46	94	17·7	86
4	12·35	77	16·73	96	18·9	91
5	19·87	124	23·8	136	22·1	106
6	15·4	96	—	—	21·1	101
7	14·8	94	—	—	—	—
Average	15·96 c.cs.		17·45 c.cs.		20·8 c.cs.	
Standard Deviation of Average, expressed as percentage of Average	5·8 per cent.		9·6 per cent.		4·3 per cent.	
Greatest Deviation (percentage)	24 per cent.		36 per cent.		20 per cent.	

in pressure. The final fall of pressure to zero, which we take as the end-point, is usually sudden. A determination should occupy not less than thirty and not more than fifty-five minutes, as has already been laid down by van Wijngaarden.

Results.

We have carried out assays of four different specimens of tincture of digitalis obtained commercially, the results are given in tabular form (see page 451), together with the standard deviation of the average $\left(\frac{1}{n}\sqrt{\frac{\Sigma\Delta^2}{n}}\right)$ and the greatest deviation of the series, both expressed as percentages of the mean.

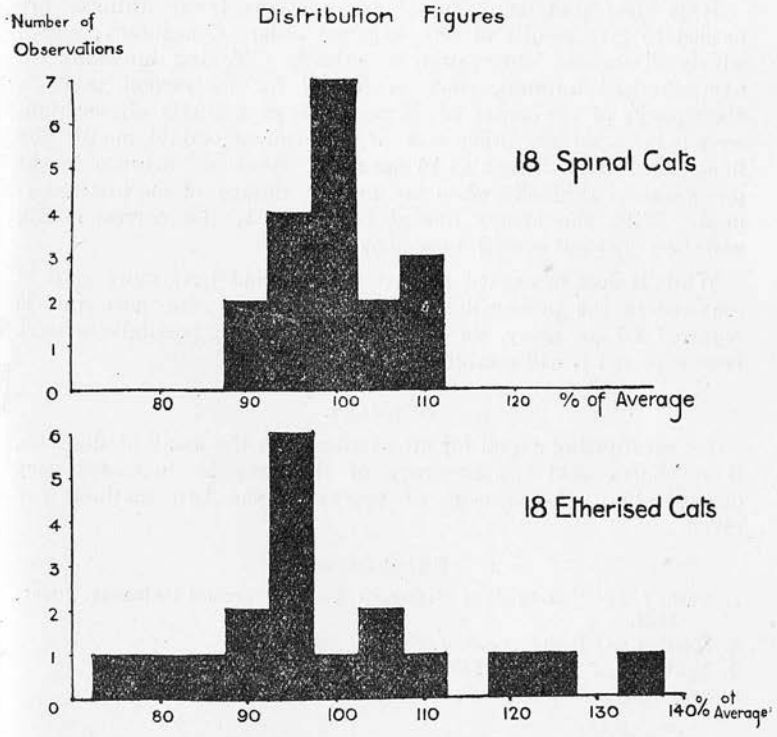

Fig. 1.

For purposes of comparison, we give a similar table prepared from results of assays made on cats anæsthetised with ether. Two of the series are taken from Burn's "Methods of Biological Assay"[1] and one is our own.

A graphical representation of these figures may be given by plotting distribution curves for equal numbers of spinal and etherised cats (Fig. 1). This is prepared by representing each cat by a square, plotting on the same ordinate all within 2·5 per cent. of the figure.

An examination of these tables will show that while the standard deviation of the average for the spinal cat is of the order of 2 per cent. and the greatest divergence of any observation in the series never exceeds 12 per cent., the corresponding figures for assays using ether are 7 per cent. and 36 per cent.

Discussion.

It is clear that using spinal preparations, fewer animals are needed to give results of any required order of accuracy, and a widely discrepant observation is unlikely. Taking an assay on five etherised animals, such as No. II in the second table, a discrepancy of the order of 36 per cent. in a single observation, were it to be on the other side of the average, would modify the final figure by as much as 14 per cent. Such a difference might prove serious clinically when the massive dosage of the tincture is used. With the assays quoted in Table 1, the corresponding variation does not exceed 4 per cent.

While it may be argued against this method that more skill is required in the preparation of the test animal and more time is required for an assay, we believe that when its possibilities have been explored it will establish its value.

Summary.

By substituting spinal for etherised cats in the assay of digitalis, it is shown that the accuracy of the assay is increased very considerably. Comparisons of assays by the two methods are given.

References.

1. Burn, J. H., "Methods of Biological Assay": Oxford University Press, 1928.
2. Hatcher and Brody, *Amer. J. Pharm.*, 1910, **82.**
3. McDowall, *J. Physiol*, 1930, **69,** 11 (*Proc. Physiol. Soc.*).
4. van Wijngaarden, *Arch. Exp. Path. Pharm.*, 1926, **113** and **114.**

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(30).

The Assay of Strophanthus Preparations by the Cat Method

By

A. D. MACDONALD

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THE ASSAY OF STROPHANTHUS PREPARATIONS BY THE CAT METHOD

By A. D. Macdonald

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Received 7th March, 1934

Introduction

When in the absence of reliable chemical tests the potency of a therapeutic agent is measured by its toxicity to animals, the reliance which can be placed on the figures obtained is limited by the variations in susceptibility to the poison which commonly occur between individuals of the species used for the test. This variation is such that, for accurate comparisons with adopted standards, large numbers of animals have to be sacrified if accuracy be important. When we compromise by using only a few animals, we must realize that a single abnormal observation may very significantly affect the assay figure, and it is desirable to make certain that such discrepancies are not due to extraneous and avoidable complications. As regards the assay of preparations of strophanthus by the cat method, evidence is presented in this paper which has been gathered with such ends in view.

In a recent paper Gaddum[1] has brought together for comparison the results obtained by nine workers in different parts of the world in estimating the strength of five given tinctures of strophanthus in terms of ouabain (the official American standard), using seven different methods. Apart from the frog methods, figures are given which are based on the toxicities to cats, rabbits, dogs and guinea-pigs. In a discussion of the relative values of the mammalian methods, it is clearly brought out that the sampling error of such tests, due to variations in sensitivity of the animals to the drug, is considerable. It is least in the case of observations by Knaffl-Lenz on guinea-pigs in which for ouabain the standard deviation of the average for a small group of animals is 4·6 per cent. The much less consistent results obtained on the cat, rabbit and dog by experienced workers lead Gaddum to doubt whether these animals will yield as consistent well-grouped figures as Knaffl-Lenz's guinea-pigs, even if the most elaborate precautions were taken as regards rate of infusion, selection of animals and so on.

Comparison of Assays on Anaesthetised and Spinal Cats

It has been shown by Macdonald and Schlapp[2] that a closer grouping of figures in the assay of digitalis by the cat method could be obtained by infusing the drug into spinal cats instead of into etherised animals. This claim has since been confirmed by Burn.[3] Before Gaddum's analysis of the relative merits of the methods for strophanthus appeared, I had extended our observations on digitalis to strophanthin, and compared the grouping of the toxicity figures for spinal and anaesthetised animals. In our earlier paper we attacked "the principle of assaying one poisonous substance upon an animal already under the influence of another." This practice is especially open to objection in the case of such a depressant as ether, which is absorbed in anaesthetically adequate but inevitably variable amounts through the lungs while the cardiac poison is infused into a vein. When a non-volatile anaesthetic is substituted for ether and administered in precise dosage according to body-weight at a fixed interval prior to the infusion, part of the objection to ether is ruled out. Knaffl-Lenz, in his assays with guinea-pigs, looked for "an anaesthetic more satisfactory than ether," and after trying and rejecting chloralose, he found urethane suitable. It is very largely to this substitution of urethane for ether, I believe, that the better grouping of his figures should be attributed, although doubtless the careful selection of the test animals and the special care in the control of the rate of the infusion as practised by this worker are contributory factors. Evidence in support of this view has been supplied by Epstein[4] who improved the grouping of figures for digitalis assays by substituting intraperitoneal paraldehyde for ether in cats, and further criticism of the use of ether will be advanced in this paper.

In Table I (pp. 184–185) I have set out assays of the toxicity of a well-known commercial brand of strophanthin (supplied as B.P.) on cats anaesthetised with ether, chloralose, and dial, and on spinal cats prepared four hours before the infusion was commenced, using the technique described in our earlier paper.[2]

The etherised animals received positive ventilation throughout and a concentration of ether just not sufficient to abolish the corneal reflex. Chloralose was administered by intravenous injection of a solution at body temperature, equivalent to 80 mgm. per kgm. after an ether induction. "Liquid Dial" was administered intraperitoneally without preliminary ether in a dosage of 0·5 mil per kgm. The infusion into animals under chloralose or dial was begun about an hour after the exhibition of the anaesthetic on the assumption that by then the animal would have achieved some sort of "steady state."

Study of the figures in Table I raises several interesting points. In the first place it is apparent that the lethal dose of strophanthin is much less for animals under ether than it is for the others. As used, ether reduces the strophanthin tolerance by about one-third. It must, then, be condemned as an accessory cause of death. The differences in toxicity to animals not under ether are not great; apparently neither chloralose nor dial as used depresses the heart to any great extent. The intermediate toxicity figure for spinal animals is surprising at first; one would expect this figure to be the highest of the four. But in its calculation no allowance is made for the fact that the head and part of the neck of the cat—at least 10 per cent. of the body-weight—are out of the circulation, since the carotid and jugular vessels are tied and the vertebrals clamped. Were this to be allowed for, the lethal dose would have to be correspondingly increased.

TABLE I

ASSAYS OF A COMMERCIAL STROPHANTHIN ON CATS UNDER ANAESTHETICS, AND ON SPINAL CATS

(1)	(2)				(3)			
Anaesthetic	Ether				Chloralose			
Experiment No.	Weight in gm.	Duration of infusion in minutes	Lethal dose in mgm./kgm.	Percentage of average	Weight in gm.	Duration of infusion in minutes	Lethal dose in mgm./kgm.	Percentage of average
1	3120	29½	0·20	87	3200	16	(0·23)	Infusion too fast
2	2640	25½	0·20	87	2450	28½	0·29	91
3	3310	48½	0·32	139	2630	33	0·30	94
4	2350	31	0·23	100	2980	36½	0·39	122
5	1950	35	0·23	100	2700	28	0·29	91
6	1560	29½	0·20	87	2900	29	0·32	100
Average lethal dose in mgm./kgm.	0·23				0·32			
Standard deviation of average	7·4 per cent.				5·2 per cent.			
Greatest deviation in the series	39 per cent.				22 per cent.			
Logarithmic variance ..	0·0062				0·0029			

TABLE I—*continued.*

	(4)				(5)			
Anaesthetic	Dial				None (Spinal Cats)			
Experiment No.	Weight in gm.	Duration of infusion in minutes	Lethal dose in mgm./kgm.	Percentage of average	Weight in gm.	Duration of infusion in minutes	Lethal dose in mgm./kgm.	Percentage of average
1	1850	27	0·31	87	2820	33	0·34	100
2	2100	30	0·35	98	3000	36	0·35	103
3	2250	31	0·38	106	2500	36½	0·39	115
4	1900	28	0·31	87	2550	29½	0·32	94
5	1920	41	0·45	126	2900	36	0·31	91
6	1980	31	0·34	95	2700	29½	0·33	97
Average lethal dose in mgm./kgm.	0·357				0·34			
Standard deviation of average	5·5 per cent.				3·2 per cent.			
Greatest deviation in the series	26 per cent.				15 per cent.			
Logarithmic variance	0·0037				0·0012			

Secondly, the grouping of the toxicity figures is distinctly closer with chloralose and dial than with ether, and best with spinal animals. In such, as previously found with digitalis infusions, the standard deviation of the average, as compared with that for etherised cats, is reduced by more than half. Gaddum, following Coward, Dyer, Morton and Gaddum,[5] for reasons which he fully discusses, recommends the use of the logarithmic variance λ^2 in such assays in place of an ordinary coefficient of variation, so this too is tabulated ($\lambda^2 = \frac{\Sigma d^2}{n-1}$ where d is the difference of the logarithm of an observation from the average of the logarithms and n is the number of observations in the series). The inference may be drawn from these figures that about twice as many etherised cats are necessary to get an assay of any required standard of accuracy as compared with cats under chloralose, and five times as many etherised as spinal cats for the same purpose!

Thirdly, it may be noted that the most divergent cat in any of these assays is one that has required considerably more strophanthin than its fellows. For the assays using ether and

dial, the worst as regards grouping, some explanation of this apparent tolerance may be offered. The normal cause of the development of the fatal issue is as follows. At the beginning of the infusion the blood-pressure slowly rises—often considerably. After fifteen to twenty-five minutes this rise is checked, and the heart becomes slow and irregular. Death usually supervenes in ten to twenty minutes, often with dramatic suddenness. Occasionally, however, the heart seems to break through this inhibition and again beats relatively fast and regularly. This third stage may last for ten or fifteen minutes more, and provided the cat dies within fifty minutes of the beginning of the infusion, as specified by the test, a discrepant figure is obtained. This third stage, apparently, is rare in spinal animals—I have not seen it in one of the thirty experiments quoted in this paper, and this probably contributes considerably to the better grouping. In the spinal cat, instead of pressure falling slowly, a sharp end-point is seen while the pressure is still fairly high. A tracing contrasting the blood-pressure end-points in spinal and anaesthetised animals is shown in Fig. 1, and described in the legend.

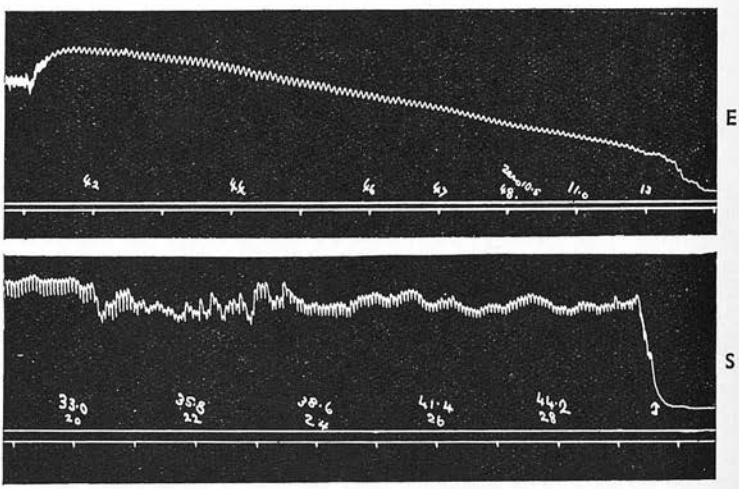

FIG. 1.—Comparison of the end-points in assays of strophanthin on etherised (E) and spinal (S) cats.

In the etherised animal the fall of blood-pressure is gradual and the end-point indefinite. In the spinal animal the pressure falls abruptly and the end-point is sharp.

It is not maintained that these tracings represent the *best* of assays on etherised and the *worst* on spinal animals, but they are reasonably typical.

The Interval between the Preparation of the Test Animal and the Infusion

Following on the appearance of Gaddum's paper, a supply of international standard ouabain was obtained through the generosity of the Director of the Department of International Standards of the National Institute for Medical Research. A solution was prepared in accordance with the official recommendations, and when assayed on spinal cats four to five hours after preparation an extraordinarily closely grouped set of figures was obtained. (Table II.) The average lethal dose in mgm./kgm. for anhydrous ouabain was found to be 0·098. Burn's figure for the average of eighteen etherised cats is 0·061, although

TABLE II

ESTIMATIONS USING SPINAL ANIMALS AND INTERNATIONAL STANDARD STROPHANTHIN POWDER

	(1)				(2)			
	Infusion 4–5 hours after preparation of animal				Infusion 2–3 hours after preparation of animal			
Experiment No.	Weight in gm.	Duration of infusion in minutes	Lethal dose in mgm./kgm.	Percentage of average	Weight in gm.	Duration of infusion in minutes	Lethal dose in mgm./kgm.	Percentage of average
1	2260	34	0·100	102	2255	20½	(0·058)	Infusion too fast
2	1750	33	0·100	102	2330	26½	0·074	97
3	2080	34	0·097	99	2700	28	0·075	98
4	1300	32½	0·094	96	2170	35½	(0·105)	Advanced Pregnancy
5	1990	28	0·092	94	1490	27½	0·071	93
6	2280	35	0·100	102	1910	29½	0·088	115
7	2460	35	0·102	104	2440	31	0·074	97
Average lethal dose in mgm./kgm.	0·098				0·0764			
Standard deviation of average	1·3 per cent.				3·4 per cent.			
Greatest deviation in the series	6 per cent.				15 per cent.			
Logarithmic variance ..	0·00026				0·0013			

TABLE II—*continued.*

(3)

Experiment No.	Infusion 7–8 hours after preparation of animal			
	Weight in gm.	Duration of infusion in minutes	Lethal dose in mg./kgm	Percentage of average
1	1510	27½	0·120	90
2	1280	38	0·154	116
3	1970	29	0·117	88
4	2010	35	0·144	108
5	1630	33	0·130	98
Average lethal dose in mgm./kgm.	0·133			
Standard deviation of average	4·8 per cent.			
Greatest deviation in the series	16 per cent.			
Logarithmic variance ..	0·0026			

we are told that "By strict adherence to the technique described by Magnus, in which the anaesthesia is lightened as infusion proceeds, and the death-point determined from the overt symptoms without record of the arterial blood-pressure, a somewhat higher lethal dose would probably be found."[7] If we remember that no correction has been made for the weight of the cat's head which has no circulation in the case of the spinal animal, the difference between these two figures is still more striking.

Clearly, ether markedly increases the susceptibility of the test animal to the toxic action of strophanthin. Now the choice of the interval of four to five hours between the preparation of the animal and the infusion of the drug was made firstly with the idea that in this period most of the ether would be eliminated, and, which is more important, the concentration left in different cats would be of the same order, and, secondly, so that two estimations might be conveniently carried out on one table in an ordinary working day. If the interval be reduced to two hours or thereabouts the grouping is less satisfactory and the toxic dose per kgm. is reduced. (Table II, col. 2.) By increasing the interval to seven or eight hours (col. 3), the toxic dose is increased but the grouping is not so good. The condition of such animals may be deteriorating by then, but they can clearly tolerate larger quantities of strophanthin. A study of Table II supplies further evidence that the toxic dose of strophanthin is probably a function of the concentration of

the ether in the test animal, thus supporting Table I in its condemnation of the use of a volatile anaesthetic.

Assay of a Tincture

In Table III I have set out an assay on a commercial tincture of strophanthin on spinal cats. Here again, apart from the first "sighting" experiment in which the infusion was too slow, there is really close grouping of the figures. Similar results have been obtained with alcoholic preparations containing other

TABLE III

Assay of a tincture of strophanthus

Spinal Cats: Infusion of a dilution of 1/500 of a tincture 4 to 5 hours after preparation of animal

Experiment No.	Weight in gm.	Duration of infusion in minutes	Lethal dose in mils/kgm. of diluted tincture	Percentage of average
1	1510	51	(20·40)	(Sighting experiment: Infusion too slow.)
2	2390	34	17·08	98
3	2410	33	16·74	96
4	2480	38	17·92	103
5	1880	41	17·73	102
6	1980	42	17·37	100
Average lethal dose in mils/kgm. ..	17·37 mils/kgm.			
Standard deviation of average	1·15 per cent.			
Greatest deviation in the series	4 per cent.			
Logarithmic variance	0·00015			

glycosides—those of digitalis, squill and convallaria. It had been thought that from such figures, coupled with the effective clinical dose of these preparations, some indication of the relative efficiency of the cardiac glycosides might be attempted, but such comparison breaks down mainly because we cannot at present readily take into account the different rates of absorption and excretion of the drugs.

Thus, according to Porter,[6] the equivalent clinical doses of strophanthin intravenously and standardised tincture of digitalis by mouth are 1/33 grain and 20 mils (1/8 mil per lb.): i.e. 1 mgm. of strophanthin is equivalent to 10 mils of tincture of digitalis.

When both drugs are given intravenously to spinal cats, 1 mgm. of anhydrous ouabain (i.e. 2·5 mgm. of strophanthin) has the approximate toxicity of 10 mils of tincture of digitalis. The discrepancy in such comparisons is doubtless related to the differences between activity following gradual absorption from the gut and the more direct action when given intravenously.

The Clinical Dose of Strophanthin

According to the British Pharmacopoeia, 1932, the dose of strophanthin, diluted so as to possess an activity which is 40 per cent. of that of anhydrous ouabain, is 0·00025 to 0·001 gm., by intramuscular or intravenous injection. According to the figures in this paper, the commercial strophanthin supplied as B.P. investigated was equivalent to 35 per cent. anhydrous ouabain. The effective clinical dose of the same preparation was found by Porter to be 1/33 grain, and this dose, administered intravenously gave a clinical result within thirty minutes similar to that which takes eight hours to develop with digitalis. She found that 1/50 grain was unreliable in its action, and 1/25 grain tended to produce toxic symptoms. Even allowing for the preparation being 5 per cent. below standard, it is clear that the Pharmacopoeial dose of strophanthin is too low, for this drug is usually reserved for emergencies in which a rapid result is required following on a single administration. The upper limit for the dose in the British Pharmacopoeia should probably therefore be doubled.

Discussion

With intravenous strophanthin there appears to be[6] but a narrow margin between the clinically effective dose (1/33 grain) and a dose producing toxic symptoms (1/25 grain) a difference of only one-third of the effective dose. Now, we have called attention to the fact that in an assay on 5 animals under ether a single discrepant but valid figure may affect the result by as much as 14 per cent.[2] which is almost half of our margin of safety. The dissatisfaction expressed by Gaddum with the grouping of the figures in assays using mammals—apart from Knaffl-Lenz's guinea-pigs—is therefore not merely academic.

In the assays on spinal animals in this paper the most discrepant toxicity figure could not affect the final result by more than 6 per cent. were it equally divergent but on the other side of the mean. Yet it will be seen from the weights of the cats used that no attempt has been made to select these animals, though there is evidence that variations in weight affect tolerance of these drugs. Further, no single experiment has been omitted in setting out these tables, and the only results excluded in the

calculations are two in which the experiment was of the nature of a preliminary "sighting" shot, and one in which the cat was advanced in pregnancy. (It is interesting to note that tolerance in this animal appears to be increased.)

Epstein's use of paraldehyde for cats or Knaffl-Lenz's urethane for guinea-pigs improves the grouping as compared with ether; the same is probably true for chloralose and dial, as shown in Table I. But Epstein admits that the adequate anaesthetic dose of paraldehyde varies widely with the age of the drug and from animal to animal. Further, he finds that under paraldehyde the lethal dose of glycoside is lower even than that found with ether, so that paraldehyde must be regarded as contributing to the death of the animal. It is hard to believe that any such contribution does not introduce an undesirable variable into an assay. The objections to spinal animals—that they take more time and possibly skill than are required with anaesthetised animals—do not seem to outweigh the closer grouping they afford.

Summary

The use of spinal rather than anaesthetised cats is recommended for the assay of the cardiac glycosides. It is shown that in the assay of strophanthin by the cat method, the grouping of the toxicity figures is improved if a non-volatile anaesthetic be substituted for the usual ether, and still further improved if spinal animals be used. Even then the toxicity figures vary according to the length of the interval between the preparation of the cat and the infusion, and it is recommended that the duration of this interval should be about four hours.

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OBSERVATIONS ON EXPERIMENTAL SHOCK

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Whilst the theory of traumatic toxaemia has been accepted in this country as providing the most reasonable explanation of the condition recognized by surgeons as secondary shock, a body of experimental research, which seems to discount this theory completely, has been produced in America during the past few years by a number of workers.[5 6 9 14] The still earlier theories have been generally discarded, since they fail to account for the diminution in the volume of the circulating blood, a diminution which Keith[1] emphasized and which is now generally admitted to be the outstanding feature of this type of shock. The theory of traumatic toxaemia arose from the observations of the Special Investigation Committee appointed by the Medical Research Council in 1917. Its reports were published officially,[2] and, later, Cannon summarized the work in his monograph,[3] in which he also shows that the other theories of the aetiology of shock are untenable.

Shock was usually produced by traumatizing one of the posterior extremities of cats. The low blood pressure which resulted from such trauma was found to occur whether or not the nerves to the limb or the spinal cord had been severed prior to the damage. The amount of swelling due to loss of plasma or blood into the traumatized area was not believed to be sufficient in itself to account for the fall in the blood pressure. Further, it was found that by clamping the main artery and vein to the limb before trauma was applied collapse of the circulation was delayed until the clamps were removed. It was assumed that a depressor substance was liberated in the damaged tissues and that the fall in blood pressure was due to the absorption of this substance into the general circulation, on which it acted.

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The Histamine Theory

About the same time Dale and Laidlaw[4] described a shock-like condition produced in animals by the injection of histamine. This condition bore so close a resemblance to the shock produced by muscle trauma that it seemed reasonable to regard the two kinds of shock as closely related. Although the pure chemical substance has been separated from many tissues of the body this histamine theory has never been substantiated by the demonstration of a depressor or shock-inducing substance in the blood of the shocked animal. Furthermore, it is quite evident, as Smith[5] points out,

> " that the failure to produce shock by crushing the muscles of a limb, the main vessels of which have been clamped, is susceptible of an interpretation other than the prevention of the hypothetical capillary poison from reaching the general circulation. It might well be supposed that shock does not occur under these conditions because there is no actual loss of blood, whereas upon removal of the clamps shock does occur because of the extravasation of blood and transudation of plasma into the lacerated tissues."

Smith showed that when blood withdrawn from a branch of the distal end of the clamped femoral vein, after the muscles of the limb had been thoroughly crushed, was returned to the circulation there was not the slightest evidence for the presence of any depressor substance in this blood. On the contrary, the blood pressure was raised, being restored to the pre-bleeding level. The transfer of such blood to another animal which had been bled but not otherwise traumatized also raised the recipient's blood pressure, and never yielded any evidence of containing histamine-like substances. If, however, histamine was injected intra-arterially into the limb the blood collected later from the clamped vein was easily shown to be depressor to the circulation of either the shocked animal or a bled recipient. Some mechanism other than the liberation of a histamine-like substance must then be responsible for the production of shock.

The Local Loss of Blood

Blalock[6] repeated the experiments of Cannon and Bayliss[7] on dogs. He found that trauma to the leg never reduced the blood pressure to a shock level without causing the loss of enough blood and plasma into the traumatized area to account for the fall. These results were opposed to the conclusions of Cannon and Bayliss, who stated: " In no case, however, was there sufficient bleeding into the wounds to account, by itself alone, for the effects observed." But Cannon and Bayliss deter-

mined the local loss of blood by weighing the extremities, traumatized and control, after amputation by symmetrical cuts across the upper ends of the thighs. Blalock soon discovered that trauma to a thigh results in extravasation into the loose tissues of the groin and flank. To include this in his comparative weighings he separated the hindquarters butcher-fashion, cutting across the body in the mid-abdominal region and splitting the lower part of the vertebral column. The difference in the weights of the limbs thus amputated in all experiments in which a low blood pressure was produced by trauma to one limb amounted to at least 4 per cent. of the body weight, or about half of the total calculated blood (taking the blood as one-thirteenth of the body weight). This work supplies such an impressive body of evidence and has received so little attention in this country that we decided to repeat, with such differences in technique as seemed advisable, the fundamental experiments of Smith[5] and Blalock.[6]

Experiments A

Depressor Substances in Blood from Damaged Limb

Dogs were anaesthetized with sodium barbitone (0.3 gram per kilo intravenously). Records of blood pressure and respiration were taken in the usual way: blood pressure, by means of a cannula in a carotid artery, connected to a mercury manometer ; and, respiration, by a tambour applied to the lower ribs. Shock was induced by striking the thigh muscles with the flat side of a 2 lb. hammer, applied so that neither the skin nor the bone was broken. For transfusion experiments clotting was prevented by the intravenous injection of chlorazol-fast pink (Boots), as recommended by Huggett,[8] in a dosage of 200 mg. per kilo body weight. By inserting a three-way cannula into the common iliac vein it was possible to withdraw either the whole or part of the blood returning from the experimental limb during or after the trauma. This gave no trouble, probably because of the efficiency of the anticoagulant. In the light of Blalock's work it is obviously desirable to draw samples from and to clamp the common iliac vessels rather than the femorals, as practised by Smith.

Sixty c.cm. of blood collected during the latter part of a shock-producing trauma were reinjected as soon as the blood pressure became steady. This raised the blood pressure, although the sample was taken during a steep fall, when the likelihood of a spread of any depressor substance present was at its greatest. In no experiment of this kind, in which we have collected and reinjected blood from a traumatized limb, have we found any evidence for the presence in that blood of any histamine-like substance, taking the blood either during the trauma or at any interval afterwards. And the same conclusion is arrived at if the blood is transferred to another animal in which no trauma has been induced and which, with

its higher blood pressure, is probably more sensitive to the action of depressor substances. If after clipping the vein we injected 1 mg. of histamine into the iliac artery of that leg and collected a small blood sample (5 to 10 c.cm.) from the vein even ten minutes later, such blood on reinjection produced a typical histamine response—a sharp fall in pressure with recovery in a few minutes.

These results are shown in the kymographic record here reproduced, and confirm Smith's claim that "direct methods have failed to demonstrate a depressor substance in the blood of the shocked animal."[5]

Experiments B

Local Loss of Blood and Plasma

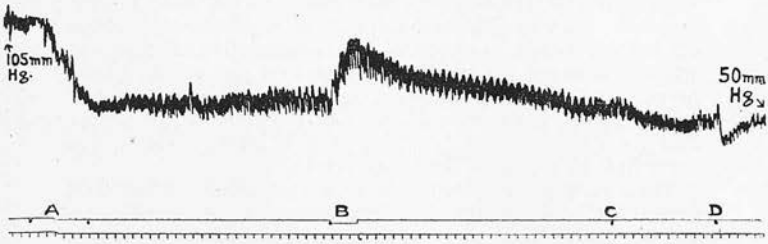
In this series of animals the same recording methods and the same technique for inducing anaesthesia and shock were used as in the previous experiment. When the blood pressure seemed to be established at a shock level the hindquarters were divided as recommended by Blalock, and weighed. The figures for ten dogs are set out in the accompanying table, which shows the blood pressure changes, the loss of blood and plasma into the traumatized area (indicated by the difference in weight between the limbs), and the proportion of the circulating blood which this loss represents. Great care was taken in dividing and weighing the limbs to ensure the reliability of these figures. The average percentage of blood volume lost into the traumatized area is calculated at 57 per cent., an ample confirmation of the observations of Blalock, who claims a corresponding figure of 50 per cent., whereas Cannon,[15] amputating at a much lower level, found that the

Experiment	Dog's Weight in Kilos	Initial Blood Pressure, mm. Hg	Shocked Blood Pressure, mm. Hg	Weight of Traumatized Limb in Grams	Weight of Control Limb in Grams	Difference in Limb Weights Calculated as Percentage of Animal's Total Blood
1	8	130	70	1,200	1,020	30
2	7½	140	85	1,240	990	43
3	6	180	50	920	580	74
4	9½	145	55	1,630	1,040	86
5	11	—	—	2,110	1,435	78
6	7	165	85	1,230	910	60
7	10½	175	55	1,910	1,450	57
8	7	150	65	1,360	1,020	63
9	9	160	40	1,570	1,250	45
10	9½	165	60	1,500	1,260	33

Average percentage of blood volume lost in traumatized area=57 per cent.

difference in weight only amounted to 11 per cent. of the blood volume. In a series of cats the corresponding figure was 43 per cent. Although lower than in dogs, this fluid loss is quite sufficient in itself to account for the collapse of the blood pressure, and makes it unnecessary to assume the absorption and general action of any histamine-like substance.

The nature of the fluid which produces the marked increase in the weight of the traumatized limb has been investigated by Blalock.[10] He found that the local gain in weight was due to the transudation of plasma rather than of whole blood, since the haemoglobin content of the fluid expressed from the traumatized limb was only 30 per cent. of that obtained from the control. This finding explains the result generally reported that the blood in traumatic shock is concentrated. We have attempted to follow the blood changes in these experiments



Female, 9½ kg. Na barbitone, 2.85 grams, as anaesthetic ; chlorazol-fast pink, 2 grams, as anticoagulant. (A) Effect of trauma on blood pressure: reduced from 105 to 60 mm. Hg ; 60 c.cm. of blood collected from the iliac vein of the traumatized leg towards the end of trauma. (B) The return of this blood raised the blood pressure from 60 to 90 mm. Hg. Within half an hour the pressure had again fallen to 60 mm. Hg. (C) The iliac vein was clamped and 1 mg. histamine injected into the left common iliac artery. (D) Twelve minutes later 6 c.cm. blood was collected from the clamped vein and injected intravenously in the other leg, producing a brief fall in blood pressure because of the histamine present.

by hourly cell counts and haemoglobin estimations. It was not unusual for the cell count and haemoglobin concentration to be increased by 25 per cent., or even more, as shock developed, but in some experiments this usual increase in blood concentration was insignificant. Whilst agreeing that there is a general tendency towards concentration of the blood, we found that the figures obtained varied with the severity of the initial shock, with the repetition of the trauma, and with the ability of the animal to compensate, with tissue fluids, for its loss of plasma.

Experiments C

The Initial Fall in Blood Pressure

In our earlier experiments we were surprised at the amount of trauma which was necessary to keep the blood pressure at a shock level. If no attempt was made to sustain the

normal body temperature shock was maintained much more readily. We found that in healthy, well-nourished animals which were kept warm the blood pressure could often be brought down to a shock level by slight trauma, but on cessation of this the blood pressure tended to rise rapidly to normal. This fall in blood pressure came on so quickly as to suggest that it was of neurogenic origin.

It has been shown by several workers that denervation of a limb, even if it includes sympathectomy[9] or transection of the cord,[3] does not prevent the development of shock following trauma. These results were obtained from experiments in which shock was maintained. We determined to investigate the cause of the initial fall in blood pressure, believing that it might produce further evidence of the changes taking place in the traumatized tissues. The animals were anaesthetized and records taken as in Experiments A and B. We found that the fall in blood pressure after mild trauma occurred: (*a*) when all nerve impulses from the limb had been cut off by means of a spinal anaesthetic, the efficiency of which was tested by faradizing the central end of the sciatic nerve ; and (*b*) when the common iliac vein was occluded, preventing the passage of blood from the traumatized tissues into the general circulation. The fall in blood pressure was prevented by occluding the iliac artery.

These findings suggested that even the initial fall in blood pressure is the result of a local change, presumably an opening up of the large vascular bed provided by the thigh muscles. Whether or not such a change is produced by the local action of a histamine-like substance as a result of the tissue injury we cannot say, but certainly, in our experiments, there is no evidence of the passage of such a substance into the general circulation.

Discussion

The old division of shock into primary and secondary types is misleading, since it does not take into account the pathological features of the two conditions. Primary shock is most reasonably explained on the basis of a disturbance of the nervous system. It is similar in origin to the fainting which not infrequently accompanies severe injuries. This variety of shock results from a reflex inhibition of the heart and a reflex relaxation of the vascular tone throughout the body. In the absence of gross injury to the nervous system there is a strong tendency to recover rapidly from this type of shock, for which the term " neurogenic " has been suggested. Secondary shock may develop several hours after injury, or in the presence of severe injuries it may follow so closely upon the primary shock that no temporary recovery is demonstrable. Any satisfactory explanation of this type of shock, for which the term " haemogenic " has been suggested, must account for the diminution of

the blood volume and the concentration of the blood which form the essential pathological features of this condition.

According to recent American work, which has been confirmed by us, the lowered blood volume is due to the loss of blood and plasma into the traumatized area. The immediate onset of this type of shock is characterized by a local vaso-dilatation of the vessels in the injured area, which is accompanied by an increased permeability of the vessel walls and a transudation of plasma into the tissue spaces. This is naturally accompanied in traumatic cases by some extravasation of blood from ruptured vessels, but, except in cases of severe haemorrhage, the loss of plasma is relatively greater than the loss of whole blood.[10] In this way some concentration of the corpuscles in the circulating blood is accounted for. The cause of the increased permeability of the capillaries in the traumatized area is not definitely known. It is probably the result of a substance which is freed locally by the injured tissues, but no clue as to its nature is obtainable, since there is no evidence of its absorption into the general circulation.

This theory of secondary shock stresses the fact, which is supported by experimental evidence, that the lowered blood pressure and blood volume so typical of this condition are due primarily to local factors and not to a general increase in capillary permeability, as postulated by Cannon and Bayliss. This local factor, though the most important, is not working alone to bring about a state of shock, for, unless the blood volume is diminished so greatly as to cause death within a short time, there is a strong natural tendency to restore the blood volume to its normal level by drawing on the fluid reserves of the body. That such a change takes place was indicated in some of our experiments by the steady rise in the blood pressure and the accompanying dilution of the previously concentrated blood which took place after the cessation of trauma. The local factor may therefore be regarded as the initiating factor, but in order to maintain the state of shock others, which may be called sustaining factors, must be present. The loss of blood which frequently accompanies injuries directly lowers the blood volume. Sweating (common in severe shock), vomiting, and, under active service conditions, the prolonged lack of food and water reduce the reserves of body fluids which are necessary to restore the blood volume. Cold and exposure are also potent influences. In the presence of one or more of these sustaining factors the blood

pressure is maintained at a level lower than is necessary to ensure an efficient circulation. Anoxaemia of the tissues, a lowered metabolic rate, increased permeability of the capillaries, and an increased viscosity of the blood are among the important changes which result from the low blood pressure. Cannon[3] sums up the situation by saying: " A series of vicious circles may thus be started which, if not interrupted, lead to a still further aggravation of the already existent abnormal state, and which account for the progressive nature of fatal shock."

Clinical Considerations

The experimental work described in this paper has been concerned with trauma applied to one of the posterior extremities of anaesthetized animals, and any clinical application must therefore be made with some reserve. In civil practice severe burns and accidents are among the commoner causes of secondary shock. We believe that our experimental work is sufficiently similar to allow of comparison with the shock found in accidents, for secondary shock is characteristically observed in connexion with extensive damage to muscles or with multiple wounds in which the sum of the damage is equivalent to considerable injury in one region. The secondary shock associated with severe burns has been somewhat overlooked as a result of the marked improvement which has followed the adoption of the tannic acid treatment. Underhill[11] claimed that the shock associated with burns was due to the marked concentration of the blood, and obtained excellent results from simply forcing fluids. Blalock[12] extended his experiments to the production of burns to wide areas of the body surface of dogs. Here again he found in the burnt and surrounding tissues an excess of fluid, akin to plasma, equivalent in amount to 40 or 50 per cent. of the total blood volume. This factor of loss of fluid after burns may be even more important in the human being than in the dog, since there is frequently a copious exudation from the injured skin in man which is not seen in dogs. It seems probable that the beneficial effect of tannic acid is largely dependent on the prevention of fluid loss as well as on lessening the absorption of toxins. Results obtained in the shock which results from intestinal manipulation[13] also stress the importance of the local factor in producing secondary shock. In fact the present outlook would seem to relegate to a secondary position the toxic factors which have been regarded for the past fifteen years as of primary importance in the production of secondary shock.

The present theory of the pathology calls for no radical change in the treatment of secondary shock. Operations under ether, chloroform, or spinal anaesthesia, which all tend to produce in the presence of a low blood volume a pronounced fall in blood pressure, are contraindicated until adequate measures have been taken to restore the blood volume to normal limits. In secondary shock the restoration of the blood volume by the slow intravenous injection of compatible blood, or, failing that, of a gum-saline solution, the application of heat to the body, and the alleviation of pain and restlessness still form the cardinal features of the therapy, but further work on the treatment of shock is now in progress.

Summary

The evidence against the acceptance of the " traumatic toxaemia " or histamine theory of secondary shock is reviewed and accepted, since: (1) we have been unable to demonstrate the presence of any depressor substance in the blood from a traumatized area ; and (2) in no experiment was the blood pressure reduced to a shock level without there being a loss of plasma and blood into the injured tissues, sufficient in itself to account for the effects observed.

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